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Alginate as Biomaterial for Tissue Engineering

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Abstract

Alginate is a naturally occurring biopolymer that due its great versatility has allowed the fabrication of a variety of biomaterials suitable for tissue engineering. Between all its different formulations, alginate hydrogels have received special attention, because they can provide an adequate three-dimensional microenvironment for tissue engineering while also providing an effective solute transport in and out of the scaffold mimicking the extracellular matrix (ECM). In this review, a systematic research of different publications related to alginate, alginate hydrogels and its current use in tissue engineering was carried out, using databases such as Google Scholar, the Norwegian University of Science and Technology Library, the Wiley Online Library and the Web of Science, without time limit or any other restriction. A total of 84 articles and original studies about alginate being used for specific tissue engineering applications were review, the vast majority were only pre-clinical trials or results for future applications, but only a few were actual clinical trials. Therefore, although great advances have been made in the study of alginate and its use as a biomaterial for tissue engineering, there is still a large gap between its researches and its future usage in humans.

Key words: Alginate, Hydrogel, Tissue engineering, Scaffold, Biomaterials, Biopolymer, Regenerative medicine.

Abstract (español)

El alginato es un biopolímero natural que, debido a su gran versatilidad, ha permitido la fabricación de una gran variedad de biomateriales para la ingeniería tisular. De entre todas sus diferentes formulaciones, los hidrogeles de alginato han recibido atención especial, debido a que pueden proporcionar un microambiente tridimensional adecuado, a la vez que permiten un transporte de solutos eficaz dentro y fuera del "scaffold" imitando la matriz extracelular (MEC). En el presente estudio, se llevó a cabo una búsqueda sistemática de diferentes publicaciones relacionadas con el alginato, los hidrogeles de alginato y su uso actual en la ingeniería de tejidos, se utilizaron bases de datos como Google Scholar, la Biblioteca de la Universidad Noruega de Ciencia y Tecnología, la Biblioteca en línea Wiley y Web of Science, sin ninguna restricción de tiempo. Se revisaron un total de 84 artículos y estudios originales sobre el uso del alginato para aplicaciones específicas de ingeniería tisular. Del total de artículos, la gran mayoría eran sólo ensayos preclínicos o resultados útiles para posibles aplicaciones futuras, y solamente unos pocos eran ensayos clínicos en fase I o II. Por lo tanto, se concluyó en que, aunque se han hecho grandes avances en el estudio del alginato y su

uso como biomaterial para la ingeniería de tejidos, sigue habiendo una gran brecha entre su investigación en laboratorios y su futuro uso en seres humanos.

Palabras clave: Alginato, Hidrogel, Ingeniería de tejidos, Andamiaje, Biomateriales, Biopolímero, Medicina Regenerativa.

1. Introduction

Alginate is a naturally occurring biopolymer extracted primarily from three species of brown algae (*Laminaria hyperborea*, *Ascophyllum nodosum*, and *Macrocystis pyrifera*) (Gombotz & Wee, 2012). In all these species, alginate is the primary polysaccharide present and it may comprise up to 40% of the dry weight (Tait, 1993). Alginate is found (in the native state) in the intracellular matrix where the polysaccharide exists as a mixed salt of various cations found in the sea water such as Mg^{2+} , Ca^{2+} , Sr^{2+} , Ba^{2+} and Na^{+} (Tait, 1993). In addition, alginate can also be isolated from two bacterial genera *Pseudomonas* and *Azotobacter* (Remminghorst & Rehm, 2006).

As biomaterial, alginate has been extensively investigated due its ability to form soft hydrogels at physiological conditions when combined with ionic cross-linking agents, such as divalent cations (Ca^{2+}) (K. Y. Lee & Mooney, 2012). Alginate hydrogels has several advantageous features including in general, good biocompatibility, non-toxic, non-immunogenic and the ability to form matrices for the microencapsulation of various molecules with minimal trauma thanks to its gentle gelling behaviour (Gombotz & Wee, 2012; Mi et al., 2002).

There are three major classes of biomaterials for tissue engineering, natural polymer, acellular tissue matrices (ceramics) and synthetic polymers (Gajendiran et al., 2017). Biomaterials provide a space for cells to form new tissues with specific structure and function, while also proving biochemical support and allowing the delivery of bioactive molecules and cells (S. J. Lee et al., 2017). As biomaterials, hydrogels have become an important field of interest because they can provide an adequate three-dimensional microenvironment and an effective solute transport in and out of the scaffold mimicking the extracellular matrix (ECM) (Slaughter et al., 2009).

Since alginate vary in chemical composition and alginate hydrogels can be prepared by different mechanism, its properties will vary depending on the type and method used for the cross-linking. Hydrogels made with alginate with a high content of guluronic acid (G) tend to have a higher mechanical stability, porosity, and tolerance to salts than those made with alginate with a low content of G blocks, which tend to form softer and more flexible gels (Strand et al., 2000).

For immobilization of living cells its preferable to have gels with high mechanical strength and great permeability to facilitate diffusion (Martinsen, Skjåk-Bræk, & Smidsrød, 1989). Therefore, with the knowledge of the chemical composition and the right choice of components and methodologies, alginate can be used as biomaterial for a wide arrange of strategies to engineer tissues (Drury & Mooney, 2003).

This study consists of and in-depth research in different scientific databases of articles and original studies related to alginate and tissue engineering. The review is structured as follows: first, the chemical structure and characterization of alginate will be described. Next, the different methods of gel formation, physical properties of the gels and other polymer dressings will be discussed. Finally, recent advances in the use of alginate for tissue engineering of specific systems such as heart, liver, pancreas, and nerve, will be given.

1. Alginates: General properties

Alginates are extracted from two main sources, algae and bacteria. Alginates commercially available are obtained primarily from algae. Some important species from which alginates are extracted: *Laminaria*, *Macrocystis*, *Ascophyllum*, *Eclonia*, *Lessonia*, *Durvillea*, and *Sargassum* (Nesic & Seslija, 2017)

Some important factors to take into account when working with alginates extracted from algae is that the composition of algae and especially the structure of polysaccharides extracted from them are under the control of enzymes, and thus often depend on the phase of life cycle, the environmental conditions, seasons, irradiation, etc. (Rinaudo, 2008). Since the cultivation of alginate is too expensive to provide alginates at a reasonable price, almost all the alginates are harvested in their wild state; therefore the quantity and quality of the alginates extracted depend on the algae species and on the season of harvest (Rinaudo, 2008). The global production of alginate is approximately 38 000 t yr⁻¹ (Helgerud et al., 2009) and almost the 30% is used in the food industry (Rinaudo, 2008). Alginates obtained from bacteria such as *Azotobacter vinelandii* and *Pseudomonas* species (Skjk-Bræk et al., 1986) are not commercially available as the ones obtained from algae but they have been studied by groups like Gorin and Spencer, Govan et al., Skjåk-Bræk, Valla, Ertesvåg, and Aachmann (Aarstad et al., 2019; Mærk et al., 2020).

1.1. Extraction and preparation

The commercial alginate extraction protocol has five steps: acidification, alkaline extraction, solid/liquid separation, precipitation and drying (Pérez, 1997). The algae are first mechanically harvested and dried, then washed and macerated. In order to precipitate alginate and at the same time extract other homopolysaccharides such as laminarin and fucoidan the extract is filtered, and treated with sodium or calcium chloride to form a fibrous precipitate of sodium or calcium alginate that can be transformed into

alginic acid by treatment with dilute HCl (Rioux et al., 2007). The quantity (between 25 and 45% dried weight of crude algae) and quality of the alginates depend on the algae species, the type and age of the tissues and methods used for extraction.

Because alginates are obtained directly from natural source, a variety of impurities may be present (e.g heavy metals, endotoxins, proteins, other carbohydrates and polyphenols), those, need to be remove before the alginate is commercially available, especially if it is going to be use for pharmaceutical applications. To address these problems, new techniques of extraction and purification have been developed and now, alginates with a greater grade of purity can be obtained from several manufactures including Kelco (Surrey, UK), NovaMatrix Biopolymer (Drammen, Norway), Chemical MFG Corp. (Gardena, CA, USA) and Junsei (Tokyo, Japan) (Gombotz & Wee, 2012).

1.2. Chemical structure and biosynthesis

Until 1950, when Fisher and Dörfel discovered the α -L-guluronic acid residues (Fischer & Dörfel, 1955), it was long believed that alginates were polymers composed of only β -D-mannuronic acid residues.

Now it's known that alginates are a family of linear block co-polymers composed of (1 \rightarrow 4)- α -L-guluronic acid (G) and (1 \rightarrow 4)- β -D-mannuronic acid (M), varying its proportions (Table 1) and sequential arrangements depending on the organism and tissue it is isolated from (Buitelaar et al., 1996).

Table 1. Chemical composition and sequence parameters for alginate. From ref (Buitelaar et al., 1996)

Alginate source	F _G	F _M	FGG	FMM	FGM/FMG	FGGG	FGGM	FMGM	NG>1
algal alginates									
<i>D. antarctica</i>	0.32	0.68	0.16	0.51	0.17	0.11	0.05	0.12	4
<i>L. japonica</i>	0.35	0.65	0.18	0.48	0.17				
<i>E. maxima</i>	0.45	0.55	0.22	0.32	0.23				
<i>A. nodosum</i>	0.39	0.61	0.23	0.46	0.16	0.17	0.07	0.09	5
<i>L. nigrescens</i>	0.41	0.59	0.22	0.40	0.19	0.17	0.05	0.14	6
<i>L. digitata</i>	0.41	0.59	0.25	0.43	0.16	0.20	0.05	0.11	6
<i>M. pyrifera</i>	0.42	0.58	0.20	0.37	0.21	0.16	0.04	0.17	6
<i>L. hyperborea</i> leaf	0.49	0.51	0.31	0.32	0.19	0.25	0.05	0.13	8
<i>L. hyperborea</i> stipe	0.63	0.37	0.52	0.26	0.11	0.48	0.05	0,07	15
Bacterial alginates									
<i>P. aeruginosa</i>	0-0.5		0						
<i>A. vinelandii</i>	0.10-0.85		0.02-0.85						

The M and G monomers are arranged in homo-polymeric regions of M and G-blocks (Figure 1), with hetero-polymeric regions of alternating structure (MG-block) (Figure 1) (Haug et al., 1967).

This block composition was demonstrated in 1966 by partial acid hydrolysis (Haug et al., 1966). During the acid hydrolysis alginic acid was separated in two fractions. One fraction was insoluble even after prolonged hydrolysis but could be divided by precipitation with acid into two fractions one containing mostly D-mannuronic acid and the other containing mostly L-guluronic acid (Haug et al., 1966, 1974). The other fraction was easily brought into solution, and a few years later was shown to consist of alternating residues of D-mannuronic and L-guluronic acid (Larsen et al., 1970).

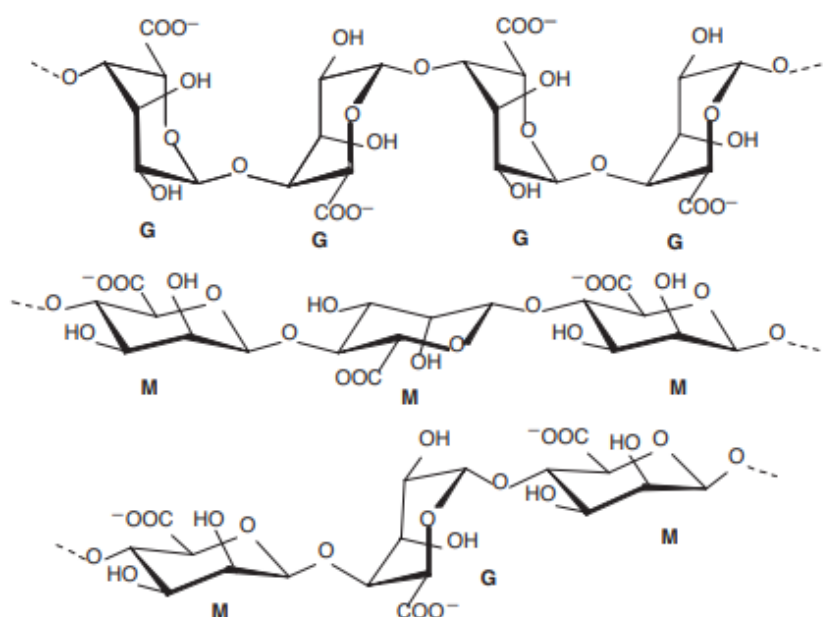


Fig. 1. Structural components of alginate: G-block, M-blocks and MG-block. (Lee & Mooney, 2012)

The properties and molecular weight of the alginate will be determined not only by the composition and length of the sequence but also by the three block type proportions (Haug et al., 1974; Smidsrød, 1974). According to some authors: “As the distribution of the monomers along the chain cannot be described by Bernoullian statics, the sequential structure is determined by measurements of diad, triad and higher order frequencies” (Buitelaar et al., 1996). The parameters in Table 1 shows the composition of the most widely used alginates determined by n.m.r spectroscopy. With the knowledge of the two monads (F_M and F_G), four diad frequencies (F_{GG} , F_{GM} , F_{MG} , and F_{MM}) and the 8 possible triad frequencies (F_{GGG} , F_{GGM} , F_{MGG} , F_{MGM} , F_{MMM} , F_{MMG} , F_{GMM} and F_{GMG}). It is possible to obtain the average block length; $N_G = F_G/F_{MG}$, and $N_M = F_M/F_{MG}$. For block consisting of at least two contiguous units equations (1) and (2) must be used (Strand et al., 2000):

$$N_{G>1} \frac{F_G - F_{MGM}}{F_{MGG}} \quad (1) \quad \text{and} \quad N_{M>1} \frac{F_M - F_{GMG}}{F_{MMG}} \quad (2)$$

As a heteropolysaccharide, alginate was originally thought to be synthesised by alternating addition of G and M residues to the growing chain (Haug & Larsen, 1969). But between 1966 and 1969 the actual biosynthetic pathway of alginate in brown algae was elucidated (Figure 2). The first steps were demonstrated in 1966 with the study of *Fucus gardneri* (Lyn & Hassid, 1966) and the final steps, which consist in the transformation of the D-mannuronic acid residues into its C(5)epimer L-guluronic acid within the polymer chain catalysed by the enzyme mannuronan C-5- epimerase (ManC5-Es), were demonstrated in 1969 (Figure 2.2) (Haug & Larsen, 1969).

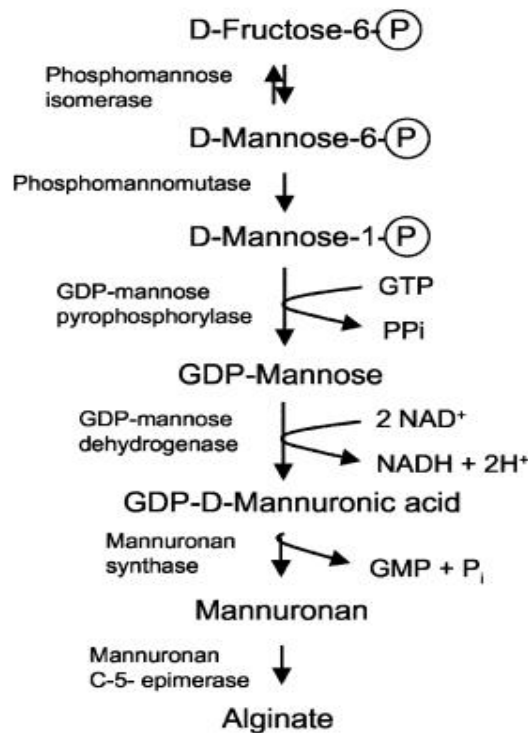


Fig. 2. Alginate biosynthesis pathway in brown algae. The first steps were demonstrated by Lin and Hassid (1966), and the final steps by Hellebust and Haug (1969). (Haug & Larsen, 1969).

Although the alginate biosynthesis mechanism has not yet been fully understood in seaweed, great advances have been made in the last years thanks to new technologies, that have allowed to identify the structural similarities between ManC5-E genes and the bacterial mannuronan C5-epimerase (Nyvall et al., 2003). In 2010 it was also described that the final steps (specifics for alginate biosynthesis) (4-6 from Figure 3) were obtained by horizontal gene transfer from Actinobacterium (Michel et al., 2010).

There are still many thing to discover about the metabolic pathway of alginate in brown algae because, there are a large number of gene products that could be involved in the production of polymers with different G and M patterns and a great number of enzymes in charge of remodelling the polysaccharides of the algal cell-wall (Fischl et al., 2016).

1.3. Molecular weight and Solubility

The molecular weight (M_w) of alginates is an important factor to take in account because it is directly related to viscosity and gelling properties. Commercially available alginates have a molecular weight that range between 3 and 500 kD. Alginates like other polysaccharides are polydisperse with respect to molecular weight, which means that the molecular weight is an average over the whole distributions of molecular weights (Strand et al., 2000).

For polydisperse polymers there are two main methods for averaging, for number-average (M_n) the equation (3) is used, in which is the total weight of the polymer divided by the total number of molecules, and for the weight-average (M_w), the equation (4) is the one used, which depends not only on the number of molecules, but also on the weight of each molecule. In a polymer mixture if N_i is the number of molecules with weight M_i and w_i is the weight fraction of polymer with molecular weight M_i these two averages can be defined as:

$$M_n = \frac{\sum_{i=1}^{\infty} N_i M_i}{\sum_{i=1}^{\infty} N_i} \quad (3)$$

$$M_w = \frac{\sum_{i=1}^{\infty} N_i M_i^2}{\sum_{i=1}^{\infty} N_i M_i} = \sum_{i=1}^{\infty} w_i M_i \quad (4)$$

By definition, the mean molar mass relation in a sample of polydisperse molecule is: $M_n < M_w$ (Koltzenburg et al., 2017).

High molecular weight alginate can be difficult to handle due to high viscosity. To address this problem, different techniques have been developed (UV-radiation, gamma-irradiation, alginate lyase,...) that allow the alginate polymer chains to be shortened to produce lower molecular weight alginates (Alsberg et al., 2003; T. Wang & He, 2010; X. Zhao et al., 2012). Low molecular weight polymer chains are currently widely studied because they present many interesting characteristics and properties such as a better antioxidant activity on oxygen free radicals (X. Zhao et al., 2012), a tightly association with other polysaccharides (oligochitosan) for the production of nanocapsules (T. Wang & He, 2010) and a faster degradation time when used as scaffolds (AlgiPharma AS, 2019; Bonino et al., 2011).

The solubility of alginates is ruled by three parameters: pH of the solvent, ionic strength of the medium and presence of gelling ions in the solvent (Pawar & Edgar, 2012).

The pKa values for the monomers of alginate (M and G) has been found to be 3,38 and 3,65 respectively (in 0,1M NaCl). Alginates pKa depends on the ionic strength of the solvent and the alginate concentration so it will differ from the monomeric residues (Haug, 1964). The abrupt decrease of pH to cause the precipitation of alginic acid from alginate solutions has been extensively studied (Haug et al., 1963, 1966, 1967).

Alginic acid is the protonized form of alginate and it is well known that is water-insoluble, even below pH 2.3 is only partially soluble. It can be slowly dissolved in solutions of sodium carbonate, sodium hydroxide and trisodium phosphate, or can react with alkali metals to give water soluble salts (alginates) (Shilpa et al., 2003).

1.4. Biocompatibility

Although the use of alginates as immobilization matrix and for the encapsulation of cells and microorganisms has been widely used, to use them for implants and tissue engineering several requirements must be met, being biocompatibility one of the most important.

The concept of biocompatibility has evolved a lot during this last decade, traditionally biocompatibility was only a concern when using implants that were going to remain for a long time. During the years 1940-1980, when the first generation of implants was being developed, using materials with low chemical reactivity was an obvious idea, because they would present a better biological performance. Due to this idea, metallic systems were replaced by stainless steels and then by titanium alloys and platinum group metals (David F. Williams, 2008). The selection of materials for implants evolved to avoid the different problems that will occur, such as corrosion, toxicity, and contamination, that triggered a biological response. Three factors caused the re-evaluation of biocompatibility because not only the composition of the material was important, other factors such as the degradation time of the material, where the implant was placed and if it reacted or not with the tissue, also had to be taken into consideration (David F. Williams, 2008).

Therefore, the concept of biocompatibility was re-defined in 1987 to:

Biocompatibility refers to the ability of a material to perform with an appropriate host response in a specific application (D. F. Williams, 1987).

Alginate biocompatibility depends strongly on the monomer content. It has been shown that alginates with low-G content induced cytokine production (Otterlei et al., 1991), but when used for encapsulation, high-G alginates showed to induce fibrotic tissue around the capsule (Tam et al., 2011). Alginate purity has been shown to influence the biocompatibility (Paul De Vos et al., 1997). However, a purification process has allowed to obtain highly purified alginate preparations, free of endotoxins, mitogens and other

contaminants (Klöck et al., 1994), allowing to minimize the importance of the alginate composition (Klöck et al., 1997). Furthermore, different studies have shown that overall, purified alginates have a higher biocompatibility than non-purified ones (Orive et al., 2005), and that chemical modifications of alginates allow to greatly reduce the fibrotic response to alginate capsules (Bochenek et al., 2018).

1.5. Alginate Derivatives

The backbone polymeric chain of alginates is composed of abundant free hydroxyl and carboxyl groups (Figure 3), which allows to do a great versatility of modifications to alter their physicochemical and biological characteristics. Some techniques used for modifications are oxidation, reductive-amination, sulfation, copolymerization and coupling of cyclodextrin units, esterification, Ugi reaction and amidations (J. S. Yang et al., 2011).

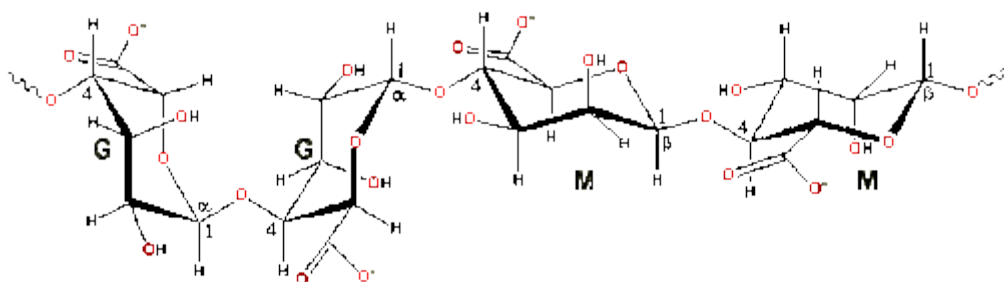


Fig. 3. Alginate backbone. (J. S. Yang et al., 2011).

1.5.1. Alginate Oxidation

The oxidation of alginate to form oxidized alginate (OA), involves the use of periodate as oxidizing agent. Periodate cleaves the C2-C3 bonds of repetitive uronic acid subunits, this leads to the formation of a dialdehyde group in the cleavage site. These aldehydes groups will spontaneously react with the hydroxyl group of the adjacent unoxidized uronic residues in the polymer chain, forming cyclic hemiacetals in equilibrium with the dialdehyde groups (Reakasame & Boccaccini, 2018). To limit side reactions the oxidation process must be carried out in complete darkness (Gomez et al., 2007).

To stop the reaction ethylene glycol must be added to the solution. The degree of oxidation can be determined using stoichiometric amounts of sodium alginate and periodate and measuring the amount of periodate consumed (B. Balakrishnan et al., 2005) or via hydroxylamine hydrochloride titration (H. Zhao & Heindel, 1991), which is based on the fact that hydroxylamine hydrochloride reacts with the aldehyde group of the OA under pH 4, generating hydrochloric (HCL). Stoichiometrically One mole of aldehyde would generate one mol of HCL. Calculating the volume of sodium hydroxide

added to maintain the pH of the mixture at 4, the amount of HCL generated can be calculated and therefore the number of aldehyde groups.

During the oxidation process, periodate also induces alginate degradation and a consequent decrease in molecular weight (X. Li et al., 2010), changes in the intrinsic viscosity and chain stiffness when the oxidation increases has also been demonstrated (Smidsrød & Painter, 1973; Vold et al., 2006). The augment in flexibility of the chains is due to the formation of the open chains adduct (Figure 4). This adduct is more sensible to hydrolytic scission, and therefore less resistant to biodegradation than alginates. This property allowed the use of partially oxidized alginates for tissue engineering applications (Bouhadir et al., 2001).

The aldehyde groups formed during the oxidation allows to do new modifications such as reductive-amination (J. S. Yang et al., 2011).

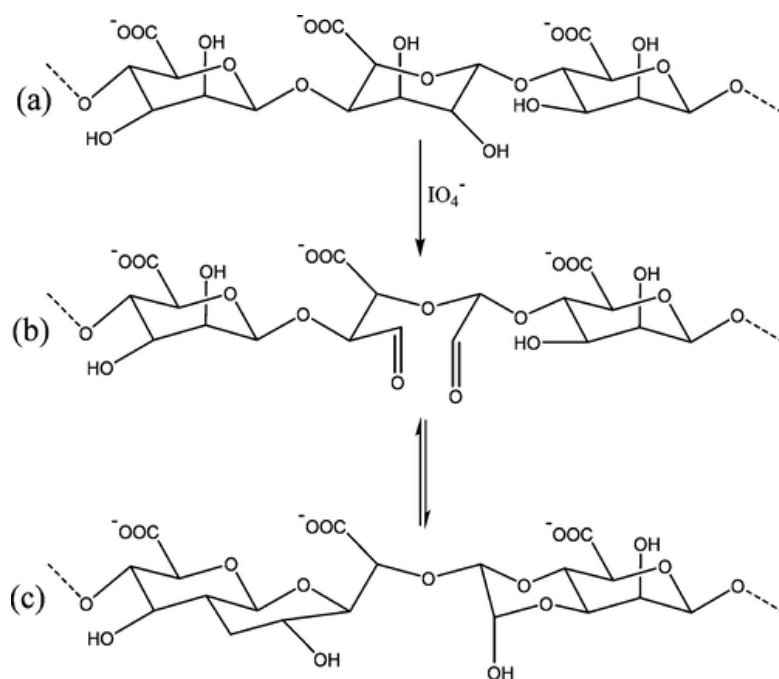


Fig. 4. Schematic representation of the Oxidative reaction of alginate by periodate. Uronic acid subunits (a), formation of the dialdehyde group (b), and formation of hemicetal (c). (Bouhadir et al., 2001).

1.5.2. Phosphorylation

Phosphorylation of alginate has been achieved recently by Coleman and co-workers using the urea/phosphate method (Figure 5) (Coleman et al., 2011), which basically consist of suspending alginate in dimethylformamide (DMF) and adding urea and phosphoric acid while stirring. The number of subunits modified with phosphate (degree of substitution) achieved with this method was limited by the heterogeneous nature of

the reaction, and varies from 5 to 26%, achieving the maximum with a molar ratio urea: H_3PO_4 :alginate of 70:20:1. The regioselectivity of the Urea/phosphate reaction with respect the four possible sites (position 2-OH and 3-OH from each residue, G and M) was studied with a detailed NMR characterization. The phosphorylation sites of the M residues were determined to occur predominantly at the M2 and M3 position with a higher regioselectivity for the equatorial 3-OH. The low amount of G residues in the sample made impossible to clearly determine its regioselectivity.

The highly acid conditions of the reactions caused a decrease in the molecular weight by a factor of 2-4. The authors pointed that hydrogels formed by blending alginate and phosphorylated alginate were more stable towards calcium extraction than those made with only unreacted alginate.

Phosphorylated alginate has been used to build fibroin-alginate beads that can be used for cell-encapsulation, and were able to support growth, proliferation of hMSCs and induced differentiation (Patil & Singh, 2019).

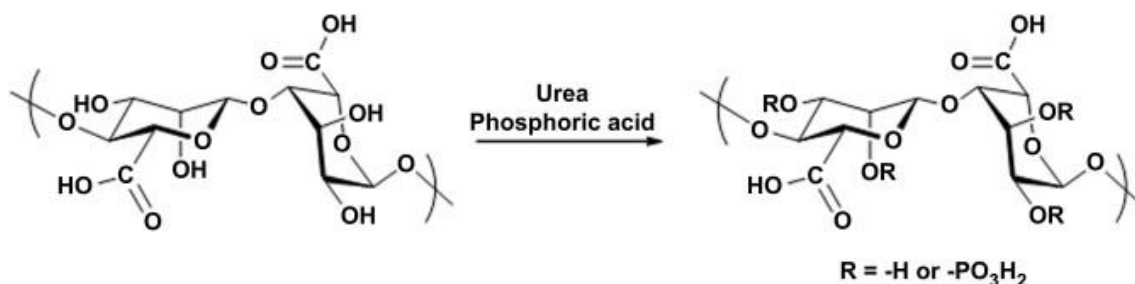


Fig. 5. Phosphorylation of alginate. (Coleman et al., 2011).

1.5.3. Sulfation of alginate

With the interest of discovering whether sulfate alginate has the same anticoagulant qualities as heparin (a natural sulfated polysaccharide with anticoagulant properties (Linhardt, 2003)), in the last decade, different ways of synthesizing sulfate alginate have been described. Being the methods most extensively used the DCC- H_2SO_4 and the ClSO_3H /formamide (Figure 6)(Freeman et al., 2008; Ronghua et al., 2003). The strong acid conditions used in both methods to achieve high degree of sulfation caused partial depolymerization and difficulties in reproducibility because of the low solubility of alginate in acid (Arlov & Skjåk-Bræk, 2017). A novel method using non acidic conditions was reported by Fan et al. (Fan et al., 2011). In this method the sulfation reagent was prepared using sodium bisulfite and sodium nitrite, and a degree of sulfation (DS) of 1.87 sulfates/monosaccharide was achieved.

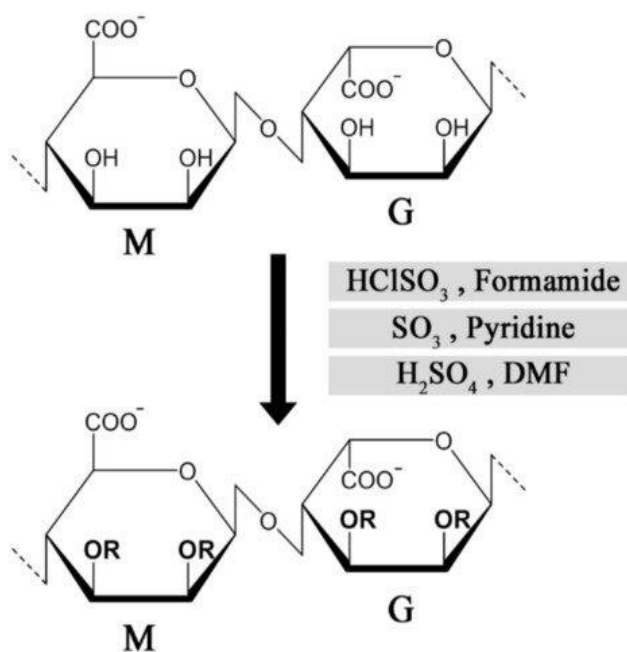


Fig. 6. Chemical sulfation of alginate using different reagents. $R=SO_3^-$ or H (Arlov & Skjåk-Bræk, 2017).

Since the substitution pattern is very heterogeneous, it's complicated to obtain detailed structural data with NMR, but some studies have demonstrated that sulfation follows a random substitution pattern and no apparent regioselectivity for the M/G or C-2/C-3 was found (Arlov et al., 2015).

The anti-inflammatory properties of sulfate alginates, has been studied recently by Arlov and Skjåk-Bræk with alginate microspheres mixed with sulfated alginate. A decrease in inflammatory cytokines was found, proving an attenuation of the inflammatory response (Arlov & Skjåk-Bræk, 2017).

Alginate sulfate has been reported to associate with multiple heparin-binding growth factors (Freeman et al., 2008). In a separate study, the relation between the ability of alginate sulfate to bind to fibroblast growth factor (FGF-2) and the degree of sulfation was found, proving that sulfated alginates are an excellent mimic of sulfated Glycosaminoglycans (GAG), which support the adhesion, proliferation and differentiation of cells (Mhanna et al., 2017). Sulfated alginate hydrogels have also been demonstrated to promote proliferation and prolong viability of chondrocytes while maintaining the cartilaginous phenotype (Mhanna et al., 2014). Due to this properties sulfated alginates have been furthered studied as a possible bioink for 3D-biorprinting, and good printability when mixed with nanocellulose and an improved retention of bone morphogenetic protein 2, osteoblastic proliferation and differentiation were found (Müller et al., 2017; Park et al., 2018).

1.5.4. Hydrophobic alginates

Alginates have a hydrophilic nature due its hydroxyl groups. Hydrophobic modification of alginate (HMA) and its characteristics have been extensively studied, and it has been proved that HMA enhance the stability of gels (Choudhary & Bhatia, 2012) and have great potential as method for controlling drug delivery systems (Choudhary et al., 2018; Colinet et al., 2009; Leonard et al., 2004; Yao et al., 2010).

The simplest method to hydrophobically modify alginate is chemical fixation, which consist in the covalent attachment of hydrophobic moieties like alkyl chains to the polymer backbone (De Boisseson et al., 2004). Because the polymer that is formed has too high molecular weight, it is impossible to characterise it by NMR without doing a partial hydrolysis. Despite this, with circular dichroism it was possible to determine that the hydrophobic alkyl chains are mostly bound on the mannuronate residues.

Jie Wu and co-workers reported a new hydrophobic modified alginate (HM-alginate) synthesized by nucleophilic substitution reaction of sodium alginate with dodecyl glycidyl ether (DGE) (Figure 7) (J. Wu et al., 2017). The HM-alginate showed a tendency to aggregate and form spherical stable micelles at a CMC 0,1mg/mL. The presence of hydrophilic and hydrophobic groups makes HM-alginate micelles suitable for the encapsulation and transport of hydrophobic drugs, improving the bioavailability (Z. Wu et al., 2020).

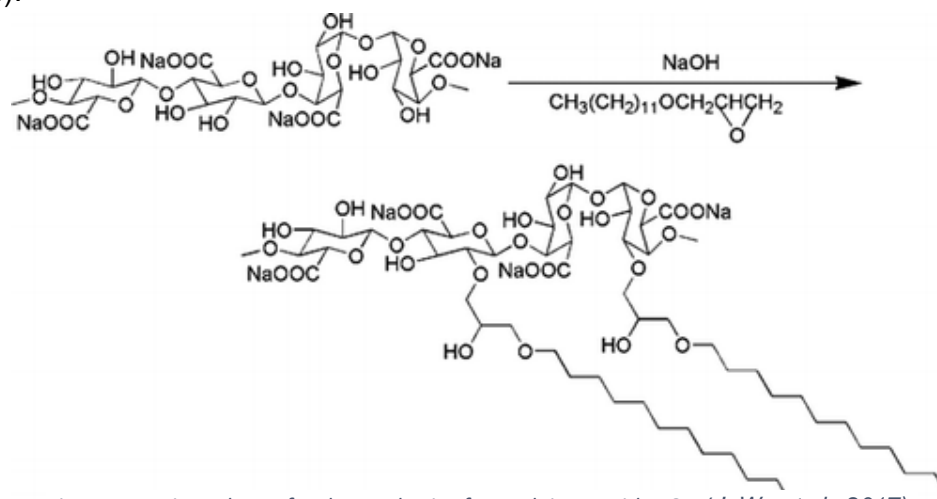


Fig. 7. Reaction scheme for the synthesis of HM-alginate with DGE. (J. Wu et al., 2017).

Zhang and co-workers reported the synthesis of hydrophobic alginate using octylamine (OA) and kaemferol (KP) (Ye et al., 2019). The analysis of the modified hydrogels (MSA/KP) showed that due to the addition of OA and KP, the MSA/KP hydrogel had better Water vapor transmission, mechanical properties and swelling than Sodium alginate hydrogels. The cell viability analysis proved that MSA/KP hydrogels were not cytotoxic and therefore, they could be used for wound healing.

2. Alginate hydrogels

Hydrogels in general, are three-dimensional networks of hydrophilic polymers with the ability to swell and retain water within its structure. The 3D-structure of hydrogels is held by physical and/or chemical cross-links between the network chains (Ahmed, 2015). The amount of water that can be absorbed by hydrogels is directly related to the osmotic pressure, capillary effect, and the presence of specific groups such as -COOH, -CONH, -OH, and -SO₃H (Patel & Mequanint, 2011). Also, their physicochemical properties depend mainly on the characteristics of the polymer, the cross-linker, the gelling environment, and the storage environment (Drury et al., 2004).

Hydrogels can be built up by two type of cross-linking junctions, namely chemical or physical. Chemical junctions like covalent interactions, are permanent, while physical junctions are transient because they arise from polymer chain entanglements or physical interactions such as ionic interaction.

Alginate hydrogels are widely used in the pharmaceutical and biomedical field as drug delivery system, wound dressing and for a wide range of tissue engineering applications (Chaturvedi et al., 2019; Gonzalez-Fernandez et al., 2016; Salehi et al., 2020).

2.1. Gelling methods

Alginate gel matrices are formed by the cross-linking of polyvalent cations with alginate in an aqueous solution. There are different mechanisms for the formation of cross-linked alginate gels: external gelation, internal gelation. For the external gelation procedure, the alginate solution is extruded into the cross-linking solution (gelling solution) where the cations would first cross-link the film surface reducing the permeability of the surface to the cations because of ion binding to the alginate, resulting in a inhomogeneous matrix because the exterior of the matrix is more cross-linked than the interior, causing a polymer concentration gradient because the alginate concentration is higher in the interface and gradually decreases towards the center of the matrix. In the internal gelation procedure, the alginate solution is mixed with an insoluble calcium salt such as Ca:EDTA and CaCO₃, to which a slow acidifier like glacial acetic acid or glucono- δ -lactone is added leading to the cross-link and the release of CO₂. The result is a homogeneous polymer distribution matrix, which may have some cavities due to the CO₂ released (Chan et al., 2006; Puguan et al., 2014; Skjåk-Bræk et al., 1989).

Both external and internal gelation methods, have their own advantages and disadvantages. Internal gelation has a longer gelation time allowing a more uniform structure and therefore a homogeneous and uniform porosity and distribution of polymer, but due to its acid conditions, it may not be viable for the encapsulation of certain

molecules or cells (Kuo & Ma, 2001). The advantages of external gelation are mainly related to its short gelation time and physiological gelling condition, these properties allows a fast and safe encapsulation of some bioactive molecules and cells (Qi et al., 2008). But as mentioned before in external gelation a polymer concentration gradient is created and a uniform distribution is hard to achieve, leaving the possibility of finding undissolved solid particles within the gel (Kaklamani et al., 2014).

2.2. Cross-linking

The selective ionic binding of alginate with alkaline earth metal ions, strongly depend on the guluronic acid content of the alginate, and can be produced with trivalent, divalent (Cd^{2+} , Co^{2+} , Cu^{2+} , Mn^{2+} , Ni^{2+} , Pb^{2+} and Zn^{2+}) and monovalent cations, although monovalent cations as well as Mg^{2+} ions, fail to produce gels due to its weak interactions with alginate (Sutherland, 1991). The divalent cations interact with blocks of guluronic acid forming ionic interactions between different chains of the polymer. As the most accepted model shows, referred to as the egg-box model, the formation of the ionic bonds, highly depends on the G-block (Sikorski et al., 2007). In the egg-box model, two polymer chains with two pairs of consecutive G units are bonded together through Ca^{2+} ions. The polymer chains adopt a three-dimensional zig-zag structure with cavities into which Ca ions are placed (Figure 8).

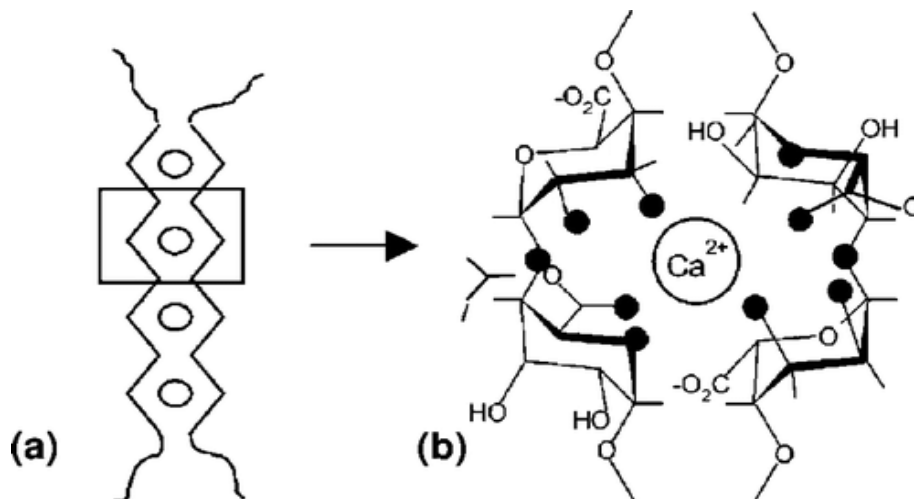


Fig. 8. Schematic representation of the egg-box model. Dark circles are oxygen atoms involved in interaction with calcium ion. (Sikorski et al., 2007).

Even though the egg-box model is the most popularly used, it is still questioned by some authors because, although the model is based on X-ray fiber diffraction, calcium alginate fibers have a very low diffraction pattern, making impossible to determine the crystal structure. Donati and co-workers reported, random GM blocks may also be involved in the junction zone and that therefore it is possible to form pure MG/MG junctions as well as mixed GG/MG junctions (Donati et al., 2005). Later, in a reexamination of the egg-model with new developed X-ray instruments. L. Li et al. reported that the junction zones of Calcium alginate gels have a 3/1 helical conformation instead of the 2/1 zigzag described in the egg-box model (L. Li et al., 2007) supporting the intervention of the MG blocks to the junction zones.

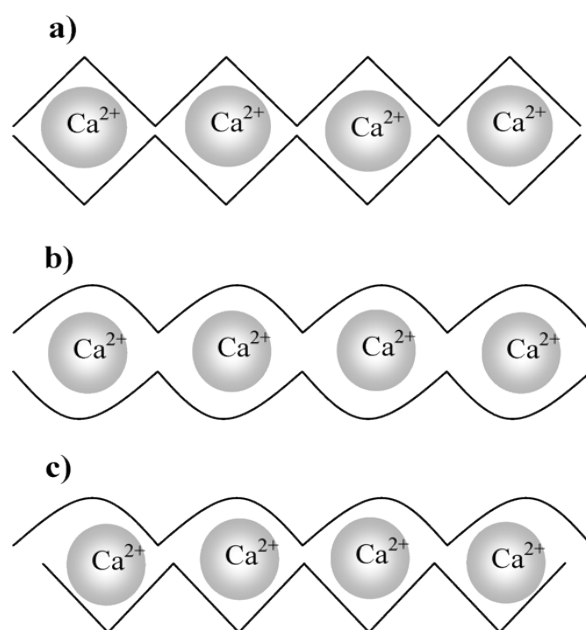


Fig. 9. Graphical description of the three possible junctions in alginate gels. (a) GG/GG junctions, (b) MG/MG junctions, and (c) mixed GG/MG junctions. (Donati et al., 2005).

Alginate can also bind to other divalent alkaline cations (Barium and Strontium) and trivalent metal ions (La^{3+} , Pr^{3+} , Nd^{3+} , Eu^{3+} , Fe^{3+} , Cr^{3+} , Al^{3+} , Ga^{3+} , Sc^{3+} , Tb^{3+}). The divalent alkaline cations bonded preferentially to GG block through ionic bonds, whereas the trivalent metal ions showed affinity for both GG and MM blocks forming strong covalent bonds (Aguilhon et al., 2012; Deramos et al., 1997). Further studies have revealed that although both divalent and trivalent cations cross-link with alginate to form gels, divalent alkaline cations exhibited a more regular structure (Brus et al., 2017). The irregularities in the trivalent cross-linked structure were exclusively ascribed to the M residues. Confirming the essential role of M-rich blocks for the promotion of alginate chains self-assembly.

In general, the structure and properties of alginate hydrogels depends on the G/M composition, alginate concentration and ion concentration (Hecht & Srebnik, 2016; Martinsen, Skjåk-Bræk, Smidsrød, et al., 1989; Russo et al., 2007).

Covalent cross-linking always alters the chemical structure of the polymer and can be produced through different techniques such as, irradiation, sulphur vulcanization or chemical reactions in conjunction with heating and pressure (Maitra & Kumar Shukla, 2014). Covalent crosslinks in addition to being chemically stable, produces an elastic structure and presents a different stress-relaxation behaviour. Some researchers have taken advantage of the covalent crosslinking relaxation method, that occurs when the water migrates out of the gel, to develop new hydrogels that permit cellular functions to occur (McKinnon et al., 2014).

Covalent cross-linked hydrogels with different degradation rates and mechanical properties have been reported (Rokstad et al., 2006). Yong Lee and co-workers reported that poly(aldehyde guluronate) (PAG) hydrogels cross-linked with poly(acrylamide-co-hydrazide) (PAH) or adipic acid dihydrazide (AAD) showed different mechanical stiffness and degradation time (K. Y. Lee et al., 2000, 2004)

The elastic substrates produced by covalent cross-linking and its stress-relaxation method, has been used to try to replicate the mechanical properties of the extracellular matrix (ECM) in alginate hydrogels, because they are thought to play an important role in regulating cellular behaviours. Modulation of the stress relaxation rate, either by coupling an oligopeptide or by covalently coupling short PEG spacer to alginate gels has proven to regulate and increase cell spreading and proliferation (Chaudhuri et al., 2015, 2016).

3. Alginate in Specific Tissue engineering systems

3.1. Alginate hydrogels for wound dressing

Even though hydrogels have a poor mechanical stability when swollen, they are still the best candidates for wound healing because they can: i) control the fluid and liquid loss, maintain wettability and moist, and ii) possess great compatibility and structure (Kamoun et al., 2017). Other wound dressing forms are films, foams and fibers and although they have their own advantages and disadvantages, they are less used.

Cross-linked alginate films are hydrophilic matrices widely used in the food industry, alginate films can be produced by immersion in a CaCl_2 solution or by directly adding CaCl_2 into the making solution. Alginate films cross-linked with calcium ions through immersion, have better water resistance, mechanical resistance, cohesiveness,

biodegradability, and rigidity than those where CaCl_2 was directly added into the film (Costa et al., 2018; Rhim, 2004; L. Z. Wang et al., 2007). For wound treatment, and especially for chronic wounds the desirable properties of the wound dressing are to provide moist and occlusion, provide protection against infections, debris, etc., and to be easily applied and removed (Thu et al., 2012). Alginate films as wound dressing are used because they have a high moisture retention and can be used as a drug-release vehicle (Dong et al., 2006). Placing one hydrocolloid film on top of another, known as by-layer films, has proven to increase their mechanical properties and water retention (Rivero et al., 2009). By-layer alginate films for the treatment of low to medium suppurating wounds have been developed using a slow-release wound healing method, loading the top layer with a drug and leaving the other drug-free to act as a control for the drug release rate (Thu et al., 2012).

Blending different components to create composite films, has shown to increase its physicochemical properties (Han et al., 2011). Rezvanian et al. has reported that an alginate-simvastatin composed film, is non-toxic for human fibroblastic cells, and has an improvement of its mechanical properties and drug-release control (Rezvanian et al., 2016). Alginate films without cross-linking have also been studied for wound dressing due its simplicity and properties when in solid state. Un-cross-linked alginate showed to be very effective in the prevention of peritoneal tissue adhesion owing to its muco-adhesive and lubricant properties (Cho et al., 2010). Therefore, un-cross-linked alginate films can be a very useful and simple adhesion barrier for the treatment of different tissue injuries.

Macroporous scaffolds as foams, have recently been acknowledge as an excellent material for regenerative medicine and tissue engineering, due its structure and properties. Alginate foams can be produced by different techniques, freeze-dry, lyophilization, and internal ionic gelation (controlled with calcium carbonate and GDL) and air-drying, the latter being one of the most recent and prominent. (Andersen et al., 2012). With internal ionic gelation and air drying it is possible to control parameters such as pore size, hydration, and mechanical integrity (Andersen et al., 2014). This flexibility in the different parameters such as alginate composition, molecular weight, mixing time, etc. allows to optimize the foam properties for any specific purpose. Alginate foams as topical drug delivery system has been studied by different authors. Hegge and co-workers, studied alginate foams as a drug delivery system with curcumin, and its applications as an antimicrobial photodynamic therapy for infected wounds (Hegge et al., 2010, 2011). Although, calcium ions are the most used divalent cations for hydrogels formation, other divalent ions may be an alternative for diverse applications. Recently a

study reported a macroporous alginate scaffold cross-linked with strontium ions and its benefits over calcium for bone tissue engineering (Catanzano et al., 2018).

3D porous scaffolds are materials that provide suitable microenvironment for cell proliferation, while also allowing the formation of new tissue and the release of biofactors such as proteins, genes (Loh & Choong, 2013). One of the problems of 3D scaffolds until recently was that they had to be surgically implanted, with injectable scaffold this problem can be overcome. Injectable scaffolds offer new advantages, such as greater ease of application, a more specific site-application and therefore a site-specific action (Biji Balakrishnan & Jayakrishnan, 2005). 3D macroporous scaffolds have been studied recently as an injectable biomaterial, the scaffolds were able to compress and rapidly recover its structure, demonstrating their potential for cell therapy with minimal invasive delivery (Bencherif et al., 2012). Additionally, 3D printing of porous alginate via three-dimensional bioplotter was achieved, with this technique, it was possible to modify various internal structures to enhance their mechanical properties (You et al., 2017).

Calcium alginate fibers were originally synthesized to be used in the textile industry, because of their properties. Fabrics made with alginate fibers were once manufactured due to their fire-resistant property, bags designed to dissolve in water were also made to transport and wash soiled hospital linen (Qin, 2008b). Currently, alginate fibers are widely used for high-tech wound dressing, due its unique properties such as non-toxicity, moisture retention and its unique gel-formation mechanism whereby, when the alginate fibers is placed in contact with wound exudates, the sodium ions in the wound exudates exchange with the calcium ions in the fibers, this exchange causes the calcium alginate fibers to turn into sodium alginate fibers, which absorbs exudates and becomes a gel that will help to retain the moisture in the wound surface (Qin, 2008a). It is also possible to process the alginate fibers into composite structures such as woven, non-woven, knitted and others, to approach specific wound problems.

Calcium alginate fibers during the ion exchange to form gels, release calcium ions that can act as a haemostatic agent, reducing blood loss from skin graft donor sites by promoting platelet activation and coagulation (Groves & Lawrence, 1986). Although they are useful for wound healing, due to their high degradation resistance, if they are not removed after haemostasis they can cause a foreign body reaction hindering the healing process (Odell et al., 1994). Alginate woven and non-woven fiber dressings have also been reported to be effective for the treatment of ulcers, reducing bacterial infections and promoting the development of healthy granulation tissue (Gilchrist & Martin, 1983). Moreover, alginate fibers can also be used as dressings for the treatment of burns

(Andrei et al., 2018). Alginate fibers are synthesised by the extrusion of an alginate solution into a second solution containing the ions for the cross-linking (Moe et al., 1994; Qin et al., 2006). This straightforward process makes the alginate fibers easy to modify by the addition of microelements, metal ions or other biologically active substance. Zinc alginate fibers, showed a higher viral protection than calcium alginate fibers and an antibacterial character that could be increased by the incorporation of bactericidal agents during fiber synthesis (Gong et al., 2011; Mikołajczyk & Wołowska-Czapnik, 2005). Since ionic silver has a broad range of antimicrobial activity, silver alginate fibers have been synthesized to explore its antimicrobial properties, the results showed that the silver ions in the alginate fibers turned the dressing from bacteriostatic to antimicrobial, because the silver ions can kill the bacteria trapped in the alginate dressing (Qin, 2005).

Recently, major efforts have been focused in developing polymeric nanofibrous scaffold because they can better mimic the extracellular matrix than the standard fibrous scaffolds. With the electrospinning technique, alginate nanofibers constructs have been achieved, but with this technique the viscosity of the solution used for the construction, must be taken in account, because only those solutions that are within a certain range of viscosity may produce fibrous structures (Bhattarai & Zhang, 2007). To solve this problem, the solution viscosity can be modified by adding synthetic polymers and surfactants. For the use of alginate nanofibers in tissue regeneration different challenges such as instability in aqueous environments and low cell-attachment needs to be approach. Leung and co-workers studied different post-electrospinning modifications to address some of these problems (Leung et al., 2014). First, to improve stability in aqueous environments and delay degradation, they did a double cross-linking with calcium and glutaraldehyde, the results showed a better degradation control in short term dressing, and better mechanical properties and stability. To improve cell attachment and biocompatibility, the scaffolds were washed and lyophilized to eliminate toxic elements from the cross-linking processes and treated with polylysine. The polylysine treatment improved cell adhesion and promoted fibroblast proliferation. Therefore, these results open the possibilities of using alginate nanofibers to regenerative medicine and tissue engineering applications.

3.2. Heart

Heart failure is one of principal causes of death in western countries with a prevalence of 23 million worldwide (Bui et al., 2011). Myocardial infarction is defined as a myocardial cell death due to prolonged ischemia caused from a permanent or temporary occlusion of the coronary arteria (Thygesen et al., 2019). The myocardium tissue has a limited

intrinsic regeneration capability since ventricular myocytes cannot be replaced once cell division ceases after birth, meaning that postnatal hearts have a fixed number of ventricular myocytes (Anversa et al., 2006). Hence, if a myocardial infarction occurs, a progressive deterioration of heart muscle function may occur due to fibrotic scar formation and left ventricular remodelling. Although a heart transplant is the best solution for myocardial failure, the low donor rate, and the inability to replace cardiac muscle loss from current techniques makes it necessary to look for alternatives (Ruvinov et al., 2008; Zammaretti & Jaconi, 2004). Biomaterials like alginate are being widely studied to overcome the standard therapies and achieve the induction of myocardial tissue regeneration.

Alginate as a material can undergo modifications with ease and has the capacity to form a scaffold in the presence of viable cells, with that in mind different techniques for myocardial tissue engineering have been developed (Dar et al., 2002). Leor et al. used a 3D alginate scaffolds seeded with partially differentiated fetal cardiac cells and reported that the cells were able to differentiate and organize into myofibers causing an attenuation in the left ventricle dilatation and hearth failure (Leor et al., 2000). Alginate cross-linking using the hydrazine-based strategy, showed adequate physical and mechanical stability, cytocompatibility and biodegradability due the covalent hydrazine link (which is easy to hydrolyse under physiological conditions), paving the way toward the creation of modular cross-linked gels that will allow an enhanced functionality and clinical applicability of myocardial tissue engineering (Dahlmann et al., 2013).

Other materials suitable to form scaffolds for myocardial regeneration, are blends of alginate with other polymers such as gelatine, which have been shown to promote myoblast cell proliferation and differentiation (Rosellini et al., 2009). In particular, designing scaffold that mimic the ECM by adding different peptides sequences are now being investigated. Attaching the arginine-glycine-aspartate (RGD) motif to alginate scaffolds, proved to promote cell attachment to the matrix and the induction of cardiomyocytes to organize into cardiac muscle tissue (Shachar et al., 2011). *In vitro*, alginate scaffolds bearing a combination of peptide motifs stemming from ECM-cell interactions, like heparin-binding peptides (HBPs) and the cell adhesion peptide G₄RGDY, have been reported to create an appropriate microenvironment for cardiac cell adhesion and growth, allowing the regeneration and assembly of cardiac muscle tissue (Sapir et al., 2011). Injectable alginate that only undergo the formation of hydrogels in the acute infarct site due the high calcium ion concentration, proved in different animal models to increase scar thickness and reduce cardiac dysfunction due to ventricular remodelling (Landa et al., 2008; Leor et al., 2009). These preclinical results,

have led to clinical investigations of intracoronary injections of alginate solutions in patients with acute MI (BioLineRx, 2009; Frey et al., 2014).

Other alginate applications involve the construction of artificial heart valves via 3D bioprinting, and although great advances have been made in this field, because of the high demanding biological and mechanical requirements of the valves, there is still very low progress in pre-clinical trials using these polymeric materials (Oveissi et al., 2020).

3.3. Bone

Bone is a complex tissue mainly composed of a hydroxyapatite, collagen, and water. The mass density-concentration of hydroxyapatite (mineral fraction) and collagen (organic fraction) depends on several factors such as age, species and organ (Hammett, 1925; Vuong & Hellmich, 2011). Bones are responsible of providing mechanical support, structural frame work, blood pH regulations and essential roles in mineral homeostasis (calcium and phosphate) (Copp & Shim, 1963; Oryan et al., 2013). Trauma, neoplasm, congenital defects, osteoporosis, and arthritis are different bone defects or fractures that may occur and need to be identified to select an appropriate treatment. Different natural bone grafts such as autograft, allograft and xenograft are available for the treatment of bone defects, but nevertheless, these grafts have a number of limitations and problems such as a limited supply, transmission of disease, and different complications due an antigenic response that makes it necessary to find new therapeutic modalities to solve the problems associated with the existing treatments. (Allison et al., 2013). The use of biomaterials for bone tissue engineering has been extensively explored due its versatility and different properties. Among many, alginate is widely used due its easy manipulation and induction of modifications to suit the principal scaffold requirements for bone tissue engineering, namely, biocompatibility, excellent porosity and pore size ($>100\ \mu\text{m}$), biodegradability, mechanical strength equivalent to cortical bone, and equal balance of osteoblastic and osteoclastic differentiation (Venkatesan et al., 2015). Modified alginates to increase cell-attachment (RGD) and degradation time, have proven to be useful as delivery systems of osteoinductive growth factors (rhBMP-2, rhBMP-4) for the treatment of critical bone defects in models *in-vivo* (Kolambkar et al., 2011; Lópiz-Morales et al., 2010). Delivering multiple factors sequentially or simultaneously to create a coordinated signal has also been studied. Co-delivering the bone morphogenic protein-2 (BMP-2) with the vascular endothelial growth factor (VEGF₁₆₅) in alginate hydrogels seeded with human bone marrow stromal cells (HBMSC) has proven to enhance the repair and regeneration of critical sized bone defects, and sequential delivery of BMP-2 and BMP-7 from alginate hydrogels promoted osteogenic differentiation of bone marrow derived

stem cells to a higher degree than with single administration (Buket Basmanav et al., 2008; Kanczler et al., 2010). Microencapsulation of cells in alginate to deliver a product when the host cells lack activity, or an accelerated healing is desired has been explored. Genetically engineered adult mesenchymal stem cells expressing rhBMP-2 under tet-regulation showed angiogenic and osteogenic activity (Zilberman et al., 2002). Co-immobilization human osteoprogenitors (HOP) and human umbilical vein endothelial cells (HUVEC) in RGD-alginate hydrogels *in-vivo*, showed an increase in mineralization in long bone defects (Grellier et al., 2009). Injectable hydrogels for bone tissue engineering are the most sought after and attractive strategies since they allow the use of non-invasive surgical procedures and a better controlled and localized delivery. Alginate mixed with inorganic polymers to enhance its mechanical strength is widely used to create gel scaffolds that replicate the bone structure. Alginate scaffolds mixed with hydroxyapatite and gelatine microspheres loading tetracycline hydrochloride showed an increase in its stability and mechanical properties, as well as a promotion of bone regeneration when osteoblast cell were encapsulated in this scaffold (Yan et al., 2016). Furthermore, injectable alginate-collagen hydrogel mixed with hydroxyapatite, has proved to promote new bone formation, a higher cell attachment and an appropriate mechanical strength as a bone tissue substitute (Bendtsen & Wei, 2015; Lin & Yeh, 2004).

3.4. Nerves, Liver, and Pancreas

Other tissues and organs that are being investigated to be regenerated with alginate gels include pancreas, liver, and nerve tissues. There are different strategies to repair central and peripheral nervous systems. Artificial nerve grafts with tubular structure are the most commonly used to guide axonal regrowth since they prevent fibrous tissue infiltration and retain neurotrophic factors (Lundborg et al., 1982). Freeze-dried alginate hydrogels covered with polyglycolic acid mesh have been used to enhance the regeneration of 50-mm gap sciatic cat nerve (Y. Suzuki et al., 1999). Alginate/chitosan hydrogels seeded with olfactory ectomesenchymal stem cells, have also been proven useful in regenerating sciatic nerve injuries in rats (Salehi et al., 2019). Non-tubular alginate-heparin gel combined with fibroblastic growth factor have also been clinically tested in patients with digital nerve injury. The results showed that the graft provided a site for nerve regeneration and complete restoration of the sensory function (Y. Suzuki et al., 2016). Spinal cord injuries were also addressed with alginate hydrogels with promising results (Kataoka et al., 2004; K. Suzuki et al., 1999). Anisotropic alginate hydrogels were introduced into axon outgrowth of rat spinal cord lesion without major inflammatory

response and directed axon regrowth (Prang et al., 2006). Alginate hydrogels seeded with syngeneic Schwann cells and a distal gradient of tet-regulated viral Brain-derived neurotrophic factor, promoted axonal regeneration and penetration into the distal spinal parenchyma (Liu et al., 2017).

The use of alginate for the microencapsulation of pancreatic islets as an effort to treat diabetes Type I was one of the first applications of alginate hydrogels. This technique allowed for successful transplantation of cells without the need of immunosuppression since the alginate microcapsules protected the graft from the host immune system (P. De Vos et al., 1997; Lim & Sun, 1980). Great advances have been made with microencapsulation technologies. Allotransplantations of microencapsulated pancreatic islets in humans without immunosuppression was achieved (Calafiore et al., 2006; Soon-Shiong et al., 1994). Although patients were unable to withdraw exogenous insulin, the insulin requirements were significantly reduced. Metabolic studies to accomplish minute-to-minute glucose levels regulation with microencapsulated islets have also been made. A study with diabetic rats in which microencapsulated islets were transplanted intraperitoneally, C-peptide instead of insulin was measured. C-peptide is released in equimolar concentrations with insulin but does not undergo hepatic extraction. With this method, due the increase of C-peptide in systemic circulation after the meal challenge, a glucose induced response of the encapsulated islets was demonstrated (Tatarkiewicz et al., 2001). Glucose clearance was also reported to be the same as non-encapsulated islets. Bioprinting is a new emerging technology that is also being explored in the creation of artificial pancreatic tissue. Even though this technology is still in development some experiments have shown that this technique has the potential to overcome some of the issues of the current techniques (Duin et al., 2019; Jia Yang et al., 2015).

Tissue engineering can provide hepatic tissue as a replacement for a failing liver. Alginate hydrogels may offer an opportunity to manufacture bioartificial livers as they have proven to promote hepatocyte cell proliferation and eliciting the formation of 3D tissue with hepatocellular functions (Dvir-Ginzberg et al., 2003). However, alginate hydrogels are mechanically unstable and lacking signals to preserve hepatocyte functions and suppress apoptosis. To overcome these inconveniences and basing on the assumption that the addition of a cell-specific ligand or extracellular signalling molecule would enhance cell-matrix and cell-cell interaction (Gutsche et al., 1996), an alginate/galactosylated chitosan hydrogel was developed, showing that this modified polymer had better stability, cell adhesion, and retention of the different functions (Chen et al., 2012; Jun Yang et al., 2001). Microencapsulating hepatic progenitor HepaRG cells in alginate, has shown to allow the self-organization of the HepaRG into spheroids, which

has been proven to be advantageous since spheroidal cultures reproduce the 3D physiological microenvironment (Pasqua et al., 2020; Rebelo et al., 2015). Furthermore, these self-assembled cells presented some of the hepatic functions (protein synthesis, enzymatic activity, biotransformation of toxins, etc.) that are within the range or above those presented in other bioartificial livers. Injectable hydrogels have also been studied as a non-invasive technique to develop three-dimensional cultures of human hepatocytes. A biodegradable and injectable hydrogel was developed, mixing alginate with glycyrrhizin and calcium. The hydrogel showed a stable structure and the ability to maintain the proliferation and liver specific function (Tong et al., 2018).

2. Objectives

The objective of this review is to make a profound bibliographic research of all the different publications related to alginate, alginate hydrogels and its current use in tissue engineering.

3. Materials and Methods

Design: A systematic review of documents from scientific societies about healthcare and medicine was carried out, in addition, other systematic reviews and scientific studies were also analyzed

Strategy: First, a general overview search was carried out in google scholar on alginate as biomaterial for tissue engineering. After that, a more specific search for the different section was made in google scholar, the Norwegian University of Science and Technology library, Wiley Online Library, and Web of Science using Boolean search with specific words such as alginate hydrogels, microencapsulation, bioprinting and alginate scaffolds, without date limit. The original scientific studies were consulted in the database of Science Direct, National institutes of Health, Springer, MDPI, ACS Publications and Nature, among others. The search was carried out using the Boolean method, using the following structure: “Alginate” AND “specific word for the tissue or organ of interest”, being the specific word “hepatocyte” for a search for studies related to liver, “myocardium” for studies related to the heart, “osteogenic” for bone studies, “nerve” for studies related to the nerve system and “pancreatic islets” for studies related to pancreas. A time limit of 10 to 15 years of publication was established. The bibliographic references of the selected articles were also reviewed in order to rescue other potential studies of interest for the review. These articles were located through Google Scholar and Scopus.

Exclusion and inclusion criteria: The main inclusion criterion applied to the scientific articles and original studies was that alginate was used as the main material in the study and that the results were conclusive and with future perspective. The exclusion criterion was that the studies were obsolete.

Data analysis: The information was structured in three sections, the first one was dedicated exclusively to alginate and its properties, the second one to hydrogels and other polymeric dressings, and the last one to specific tissue engineering applications using alginate.

4. Results and Discussion

Table 2 of the Annex I, displays all the articles and original works used to carry out the thesis, collected and classified according to their Authors, Title, Journal or Booktitle, Volume, Number and Year.

Table 3 shows the distribution of the articles used in the main sections of the review.

Table 3. Distribution of articles in the main sections of the review.

Total	Introduction	Alginate: General properties	Alginate Hydrogels	Alginate in Specific Tissue Engineering Systems
195	7	74	29	85

In the Table 4, the number of articles used within each subsection of the three main sections of the review are displayed. This table also represents the evolution of the number of articles and how the process of selecting them has advanced.

Table 4. Number of articles in each main section and subsection.

Main section	Subsections					
Alginate: General properties	Extraction and preparation	Chemical structure and biosynthesis	Molecular weight and Solubility	Biocompatibility	Derivatives	
74	3	14	10	9	30	
Alginate Hydrogels	Cross-linking	Gelling methods				
29	16	7				
Alginate in Specific Tissue engineering systems	Wound dressings	Heart	Bone	Nerve	Pancreas	Liver
85	32	16	15	8	7	7

The Figure 10 shows a graphic representation of the distribution and number of articles used in the main sections of the review.

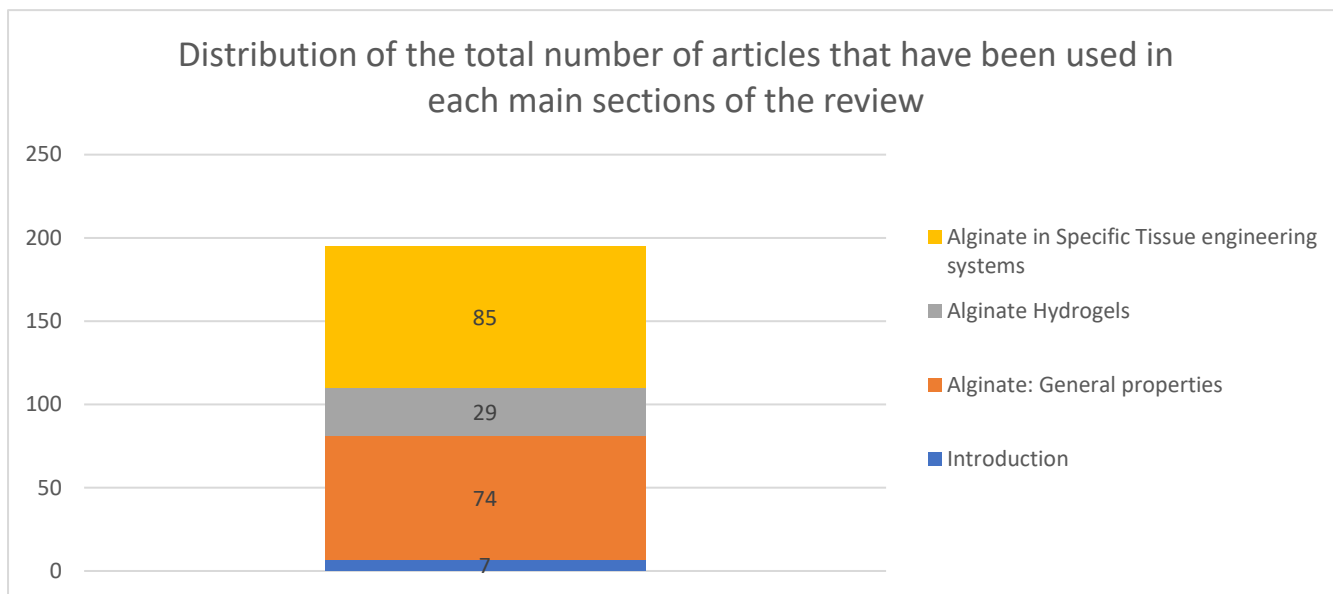


Fig. 10. Distribution of the total number of articles that have been used in each main sections of the review.

The Figure 11 represents the number of articles used in each subsection of the main section “Alginate: General properties”. It also represents the number of articles found and selected when using the search structure: “Alginate” [AND] “keyword”, being the keywords: Chemical structure, Composition, Biocompatibility, Derivatives, Oxidation, Sulfation, Phosphorylation and Hydrophobic.

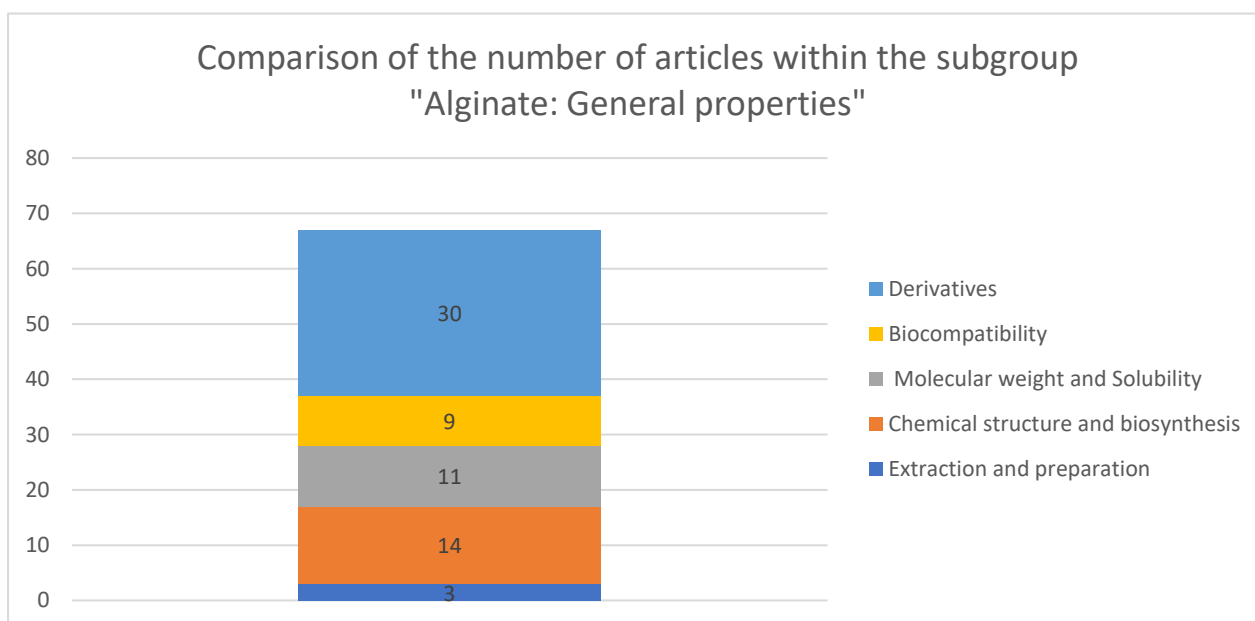


Fig. 11. Comparison of the number of articles within the subgroup "Alginate: General properties".

The Figure 12 represents the number of articles used in each subsection of the main section “Alginate Hydrogels”. It also represents the number of articles found and included in the review when the following search structure was used: “Alginate Hydrogel” [AND] “keyword”, being the keywords: Gelation, Gelling methods, Cross-linking, and ionic gels.

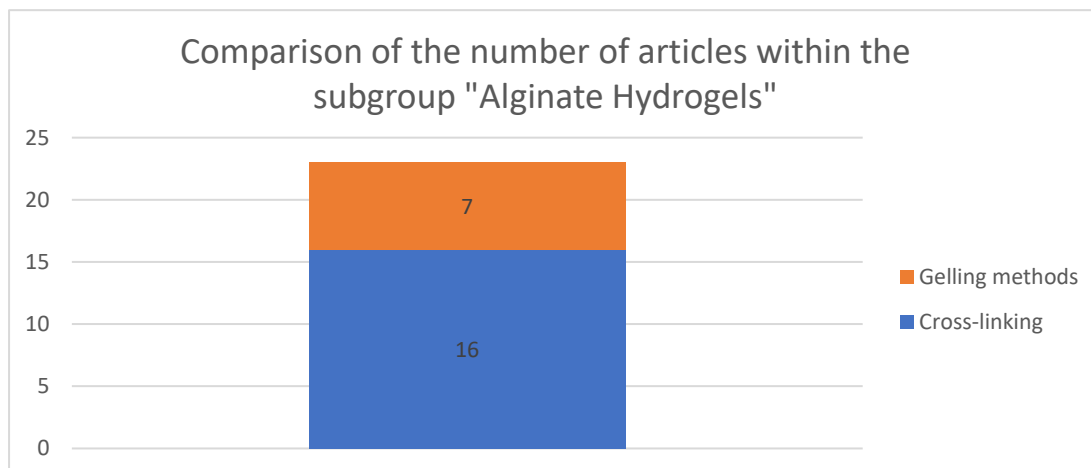


Fig. 12. Comparison of the number of articles within the subgroup "Alginate Hydrogels".

The Figure 13 represents the number of articles used in each subsection of the main section “Alginate in Specific Tissue Engineering Systems”. It also represents the number of articles and original studies selected and included in the review when the following search structure was used: “Alginate” [AND] “keyword”, being the keywords: Scaffolds, Nerve, Heart, Liver, Pancreas, Bone, and Wound dressing.

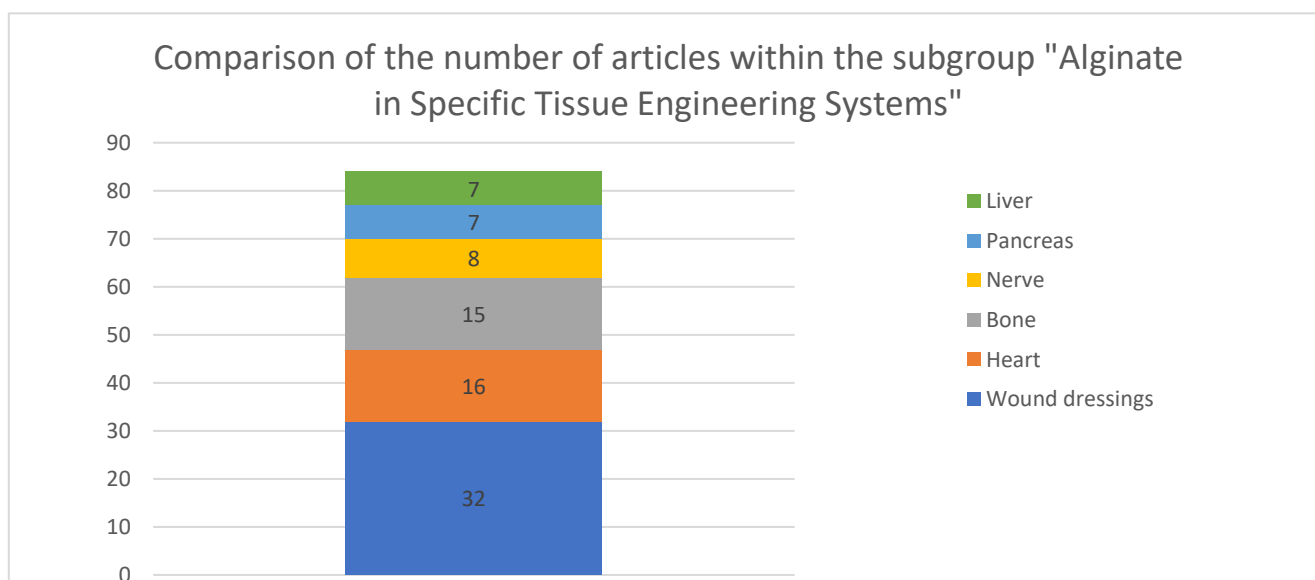


Fig. 13. Comparison of the number of articles within the subgroup "Alginate in Specific Tissue Engineering Systems".

The table 5 shows all the articles and original studies used to complete the subsection of the review “Alginate for Wound dressing”. The table is organized according to their research group, year of publication and a summary of the most relevant results.

Table 5. Articles used in the section Alginate for Wound dressing.

Research Group	Year of publication	Summary
Gilchrist, T.; et al.	1983	Highly absorbent biodegradable dressing derived from seaweed that can be successfully applied to cleanse a wide variety of secreting lesions. The high absorption of exudate is achieved via strong hydrophilic gel formation and this serves to control wound secretion levels and to minimize bacterial contamination.
Groves, A. R.; et al.	1986	Use of an alginate fibre dressing to reduce blood loss from skin graft donor sites. Significant haemostasis was achieved in the immediate post-surgery phase and no adverse reactions observed.
Moe, Størker; et al.	1994	Calcium alginate gel fibers prepared with an inhomogeneous polymer concentration profile showed higher moduli than did fibers with a homogeneous polymer concentration profile. The higher modulus observed for fiber prepared from an alginate with low Ca ²⁺ affinity is tentatively explained as resulting from a higher degree of polymer chain alignment.
Odell, E. W.; et al.	1994	Report of a florid foreign body giant cell reaction elicited by Kaltostat (wound dressing made from non-woven sodium calcium alginate fibres) which had been used to obtain haemostasis in an apicectomy cavity on an upper lateral incisor approximately 7 months earlier. The case demonstrates that alginate fibres left in situ may elicit a long-lasting and symptomatic adverse foreign body reaction.
Rhim, Jong Whan	2004	Measuring the properties of sodium alginate films, when modified using two different methods of CaCl ₂ treatment, i.e. the direct addition of CaCl ₂ into film making solution (mixing films) and the immersion of alginate films into CaCl ₂ solutions (immersion films).
Balakrishnan, Biji; et al.	2005	Report of a new class of hydrogels derived from oxidized alginate and gelatin. Periodate-oxidized sodium alginate had appropriate molecular weight and degree of oxidation, rapidly cross-links proteins such as gelatin in the presence of small concentrations of sodium tetraborate (borax). The gel was also found to be biocompatible and biodegradable. The results proved the potential of the system as an injectable drug delivery vehicle and as a tissue-engineering scaffold is demonstrated by using primaquine as a model drug and by encapsulation of hepatocytes inside the gel matrix, respectively.
Mikołajczyk, Teresa; et al.	2005	Development of conditions for the manufacture of fibres designed for medical applications from zinc alginate and copper alginate. The high moisture absorption and anti-bacterial effects of fibres from zinc or copper alginate allowed the production of a new generation of dressing materials. At the same time, the tenacity of copper alginate fibres made possible to obtain flat textile materials designed for medical application.
Qin, Yimin	2005	Study that incorporated silver ions into alginate fibres, resulting in a highly absorbent alginate wound dressings with antimicrobial properties. Laboratory studies proved that these fibres were highly effective against bacteria.
Dong, Zhanfeng; et al.	2006	Obtention of alginate and gelatin films, cross-linked with Ca ²⁺ , with ciprofloxacin hydrochloride as model drug incorporated in different concentrations, via casting/solvent evaporation method. The results of controlled release tests showed that the amount of ciprofloxacin hydrochloride released decreased with an increase in the proportion of gelatin present in the film.
Qin, Yimin; et al.	2006	Use of hydrochloric acid to convert calcium alginate fibers into alginic acid fibers, which were further converted into sodium alginate fibers by treating the fibers with sodium hydroxide in organic solvent. Results showed this method improved gel blocking properties and absorption capacities of the fibers.
Bhattarai, Narayan; et al.	2007	Fabrication of natural polymer alginate-based nanofibers by electrospinning blend solutions of alginate and polyethylene oxide (PEO). The results proved that polymer solution viscosity is a key factor that regulates the electrospinnability of the solution and the structure of the electrospun product. This study also revealed that the polymer solution properties and thus the solution spinnability changed over storage time in the ambient environment.
Wang, Lizhe Z.; et al.	2007	This study assessed the film-forming abilities of six types of proteins, as well as six types of polysaccharides at various concentrations. Biopolymer films evaluated included: sodium caseine (SC), whey protein isolate (WPI), gelatine (G); caboxymethyl cellulose (CMC), sodium alginate (SA) and potato starch (PS). Screening trials showed that optimal film-forming conditions were achieved using SC and G (4% and 8%), WPI (8% and 12%), PS, CMC (2% and 3%) or SA (1% and 1.5%) solutions heated to 80 °C in combination with 50% (w/w) glycerol. Films manufactured from 1.5% SA, 8% G and 3% CMC had the highest tensile strength, flexibility, tear strength, and puncture resistance, respectively.
Qin, Yimin;	2008	Review of the principles gel-forming process for alginate fibers and analyzed the gelling behaviour of various types of alginate fibers.
Qin, Yimin	2008	Review of the development in the production of various fibers from alginate, and summary of the production processes for calcium alginate, calcium/sodium alginate, sodium alginate, zinc alginate, silver alginate and other types of alginate fibers containing novel functional ingredients.

Table 5. Part II		
Research Group	Year of publication	Summary
Rivero, S.; et al.	2009	Development of composite, bi-layer and laminated biodegradable films based on gelatin and chitosan, determination of its film barrier and mechanical properties and characterization of their microstructure.
Cho, Wan Jin; et al.	2010	Study about un-cross-linked alginate as a physical tissue adhesion barrier material. The animal test results, demonstrated that the un-cross-linked alginate film was more effective for the prevention of peritoneal tissue adhesion than other types of alginates. It also showed a low inflammatory response and did not lead to specific histological influence during the wound healing.
Hegge, Anne Bee; et al.	2010	Incorporation of a model water-insoluble photosensitizer, curcumin, in novel alginate foams, to evaluate the suitability of the curcumin loaded foams in antimicrobial photodynamic therapy of infected wounds. The release of curcumin in its monomeric form was demonstrated in vitro and found to be dependent on the type and amount of cyclodextrins in the formulation.
Gong, Ying; et al.	2011	Investigation of the cytotoxicity and anti-influenza virus (IFV) activity of calcium or zinc alginate fibers. cultured with alginate fibres were used to screen cytotoxic effects. The results showed that calcium or zinc alginate fibers had a good cellular biocompatibility with the African Green Monkey kidney cell (Vero) and human cervical cancer cell (Hela). Furthermore, the large weight zinc alginate fibers had a better anti-IFV activity than calcium alginate fibers.
Hegge, Anne Bee; et al.	2011	Curcumin loaded alginate foams were proposed for application in antimicrobial photodynamic therapy of infected wounds. The foams remained intact after hydration and would be possible to remove from the wound prior to irradiation without causing any tissue damage. Cyclodextrins (CDs) and polyethylene glycol 400 (PEG 400) were selected as solubilizers of curcumin in the foams to provide a burst release of the photosensitizer. The results showed a reduction of <i>Enterococcus faecalis</i> cells and a reduction in the viability of <i>E. coli</i> cells.
Han, Donling; et al.	2011	Investigation of the structure, thermal stability and mechanical properties of the composite films by wide-angle X-ray diffraction, Fourier transform infrared spectroscopy, scanning electron microscopy, atomic force microscopy, thermogravimetry analysis, and mechanical test. The results obtained from those different studies revealed that chitosan and graphene oxide could mix with each other homogeneously and the mechanical properties of the prepared films were improved significantly over that of the pure chitosan film.
Andersen, Therese; et al.	2012	Presentation of a method to prepare alginate-based foams, based on homogeneous, ionotropic gelation of aerated alginate solutions, followed by air drying. The method allowed a higher flexibility and better control of the pore structure, hydration properties and mechanical integrity.
Bencherif, Sidi A.; et al.	2012	Description of a strategy to deliver via conventional needle-syringe injection large preformed macroporous scaffolds with well-defined properties. The resulting gels demonstrated the capability to withstand reversible deformations at over 90% strain level, and a rapid volumetric recovery long-term and the release of biomolecules in vivo.
Thu, Hnin Ei; et al.	2012	Development of a novel bilayer hydrocolloid film based on alginate and the investigation of its potential as slow-release wound healing vehicle. The characterisation results showed that bilayer has superior mechanical and rheological properties than the single layer films. The bilayers also showed low MVTR, slower hydration rate, lower drug flux in vitro and a significant higher healing rate in vivo, with a well-formed epidermis and faster granulation tissue formation when compared to the controls.
Loh, Qiu Li; Choong, Cleo	2013	Review of the various fabrication techniques that have been employed to fabricate 3D scaffolds of different pore sizes and porosity.
Andersen, Therese; et al.	2014	Development of a new and flexible method for preparation of dry macroporous alginate foams with the capability of absorbing physiological solutions. The study demonstrated how the gelation rate of the alginate and degree of ionic crosslinking can be utilized to control the physical foam properties. The method of preparation of such foams allows, tailoring of the pore structure, hydration properties and mechanical integrity in a manner not possible by other techniques.
Leung, Victor; et al.	2014	This study addresses several challenges in alginate nanofiber application, including the lack of structural stability in aqueous environment and limited cell attachment, via examining crosslinking techniques. The results showed that with optimization of the electrospinning solution, nanofiber morphology was maintained after the two-stage crosslinking process. The aqueous stability and cell attachment also improved after the postspinning modifications.
Rezvanian, Masoud; et al.	2016	Previously, studies have demonstrated that topical application of simvastatin can promote wound healing in diabetic mice via augmentation of angiogenesis and lymphangiogenesis. Formulation and characterization of simvastatin in alginate-based composite film wound dressings. The in vitro drug release results, revealed that alginate/pectin film produced a controlled release drug profile, and the cell viability assay showed that the film was non-toxic.
Kamoun, Elbadawy A.; et al.	2017	Review of the present, past and current efforts of hydrogel membranes fabrication from biopolymers and synthetic ones for wound dressing applications.

Table 5. Part III

Research Group	Year of publication	Summary
You, Fu; Wu, Xia; Chen, Xiongbiao	2017	Use of a three-dimensional biplotting technique supplemented with thermal/submerged ionic crosslinking process to fabricate hydrogel scaffolds. Results demonstrated that the mechanical performance of hydrogel scaffolds can be tuned by changing the internal structure parameters including strands orientation and spacing between strands.
Andrei, Mihaela-Cristina; et al.	2018	Study of appropriate burn wound management, its multiple treatment modalities and the specific sequencing particular to each patient, addressing every injured area depending on burn surface and depth, patient's general status and the available infrastructure and burn center resources. Ongoing and future researches into the healing of burn wound are also discussed.
Catanzano, Ovidio; et al.	2018	Development of macroporous alginate foams (MAFs) with porous and well interconnected structure, to enhance growth and osteogenic differentiation of human Mesenchymal Stem Cells (hMSCs). This study also reported a new method for MAFs fabrication based on the combination of internal gelation technique with gas foaming. The biological assays showed how scaffolds with high strontium content are able to support cell growth and differentiation in long times by promoting osteogenic marker expression.
Costa, Maria J.; et al.	2018	Cross-linking of alginate-based films with different (M/G) ratios to fully understand the effect of calcium chloride (CaCl ₂) crosslinking and the mannuronic (M) and guluronic (G) acid ratio (M/G) of alginate structure in the films properties. The results showed that the crosslinking significantly affected the alginate structure and properties, decreasing film thickness, moisture content, solubility and water vapour permeability, and also proved the relation between M/G ratios and CaCl ₂ concentrations and the resulting film's properties.

The table 6 shows all the articles and original studies used to complete the subsection of the review “The use of alginate for heart tissue engineering”. The table is organized according to their research group, year of publication and a summary of the most relevant results.

Table 6. Articles used in the section Alginate for Heart tissue engineering.

Research Group	Year of publication	Summary
Leor, Jonathan; et al.	2000	After isolating and growing fetal cardiac cells within 3D porous alginate scaffolds. Rats with myocardial infarction were randomized to biograft implantation or sham-operation into the myocardial scar. Alginate scaffolds provided a conducive environment to facilitate the 3D culturing of cardiac cells and after implantation into the, the biografts stimulated intense neovascularization and attenuated LV dilatation and failure in the experimental rats.
Dar, Ayelet; et al.	2002	Cell seeding within porous alginate scaffolds, applying a moderate centrifugal force during cell seeding to achieve uniform cell distribution throughout the alginate scaffolds, and consequently enabling the loading of a large number of cells onto the 3D scaffolds. The highly dense cardiac constructs maintained high metabolic activity in culture. Some of the cell aggregates contracted spontaneously within the matrix pores.
Zammaretti, Prisca; Jaconi, Marisa	2004	Overview on some of the most promising materials and cell-therapy strategies used in the past few years for the regeneration of the wounded heart.
Anversa, Piero; et al.	2006	Discussion of the current controversy about the role that endogenous and exogenous progenitor cells have in cardiac homeostasis and myocardial regeneration following injury. Favoring the notion that the mammalian heart has the inherent ability to replace its cardiomyocytes through the activation of a pool of resident primitive cells or the administration of hematopoietic stem cells.
Landa, Natali; et al.	2008	A novel absorbable biomaterial composed of calcium-crosslinked alginate solution, which displays low viscosity and, after injection into the infarct, undergoes phase transition into hydrogel was developed. The beneficial effects were comparable and sometimes superior to those achieved by neonatal cardiomyocyte transplantation. Showing for the first time that the injection of in situ-forming, bioabsorbable alginate hydrogel is an effective acellular strategy that prevents adverse cardiac remodeling and dysfunction in recent and old myocardial infarctions in rat.
Ruvinov, Emil; et al.	2008	Review of recent advancements in cardiac cell, gene-based, tissue engineering therapies and a selected strategy in cell therapy and new tools for myocardial gene transfer are summarized.

Table 6. Part II

Research Group	Year of publication	Summary
Leor, Jonathan; et al.	2009	Develop of a calcium cross-linked alginate solution that undergoes liquid to gel phase transition after deposition in infarcted myocardium. The Intracoronary injection of alginate biomaterial was feasible, safe, and effective.
BioLineRx, L	2009	Phase I, multi-center, open label study designed to assess the safety and feasibility of the injectable BL-1040 implant to provide scaffolding to infarcted myocardial tissue.
Rosellini, Elisabetta; Cristallini, Caterina; Barbani, Niccoletta; Vozzi, Giovanni; Giusti, Paolo	2009	Preparation of blends based on alginate and gelatin, with different weight ratio, to combine the advantages of these two natural polymers for application in cardiac tissue engineering. The results showed a better stability of the blends in cell culture medium than in PBS and suggested a mainly hydrolytic degradation process. Cell culture tests, showed a good cell proliferation for all the blends containing more than 60% of gelatin, with the alginate/gelatin 20:80 showing the best response.
Sapir, Yulia; Kryukov, Olga; Cohen, Smadar	2011	Combination of two matrix-attached peptides, the adhesion peptide G4RGDY and heparin-binding peptide G4SPRRARVITY (HBP) for cardiac tissue regeneration using alginate scaffolds seeded with neonatal rat cardiac cells. The cardiac tissue developed in the HBP/RGD-attached scaffolds revealed the best features of a functional muscle tissue. Also showing the preservation and an increase in Connexin-43 expression (Cx-43), and the formation of a contractile muscle tissue in the HBP/RGD-attached scaffolds.
Shachar, Michal; Tsur-Gang, Orna; Dvir, Tal; Leor, Jonathan; Cohen, Smadar	2011	Role of matrix attached RGD peptide in the engineering of cardiac tissue within macroporous scaffolds. Neonatal rat cardiac cells were seeded into RGD-immobilized or unmodified alginate scaffolds. The immobilization of RGD peptide into macroporous alginate scaffolds promoted cell adherence to the matrix, prevented cell apoptosis and accelerated cardiac tissue regeneration proving to be a key parameter in cardiac tissue engineering, contributing to the formation of functional cardiac muscle tissue and to a better preservation of the regenerated tissue in culture.
Bui, Anh L.; et al.	2011	Discussion of the key features of the epidemiology and risk profile of Heart Failure.
Dahlmann, Julia; et al.	2013	Development of a fully defined in situ hydrogelation system based on alginate (Alg) and hyaluronic acid (HyA), in which their aldehyde and hydrazide-derivatives enable covalent hydrazone cross-linking of polysaccharides in the presence of viable myocytes. The hydrogel allowed for the generation of contractile bioartificial cardiac tissue from CM-enriched neonatal rat heart cells, which resembles native myocardium.
Frey, Norbert; et al.	2014	Clinical trials of an alginate solution (IK-5001) using Patients (n=27) with moderate-to-large ST-segment-elevation myocardial infarctions. IK-5001 was administered by selective injection through the infarct-related coronary artery. The results showed favorable tolerability of the procedure and the preservation of left ventricular indices and left ventricular ejection fraction.
Thygesen, Kristian; et al	2019	Definition of myocardial infarction according to the European Heart Journal and the journal of the American college of cardiology.
Oveissi, F.; Naficy, S.; Lee, A.; Winlaw, D. S.; Dehghani, F.	2020	Discussion of the current and potential materials that can be used for developing of artificial heart valves along with the existing and developing fabrication methods. It also compares the mechanical properties of various materials that are currently used or proposed for heart valves along with their fabrication processes to identify challenges when creating new materials and manufacturing techniques to better mimic the performance of native heart valves.

The table 7 shows all the articles and original studies used to complete the subsection of the review “The use of alginate for bone tissue engineering”. The table is organized according to their research group, year of publication and a summary of the most relevant results.

Table 7. Articles used in the section Alginate for Bone tissue engineering.

Research Group	Year of publication	Summary
Hammett, Frederick S.	1925	Study of the chemistry of bone growth, extending the picture to include the differential development of bone with respect to calcium, magnesium, and phosphorus incorporation.
Copp, D. Harold; Shim, S. S.	1963	Study of the regulation of the calcium levels in plasma and the function of the bone as an ionic homeostasis factor and a long-range mineral control. Proving that excess calcium and phosphate may be stored in bone or that when the levels of calcium or phosphate are low these elements may be mobilized to maintain the essential level, resulting in a loss of bone mineral.
Zilberman, Yoram; et al.	2002	Development of a technique to efficiently encapsulate engineered AMSCs within polymeric alginate microcapsules, with maintained viability, differentiation by autocrine effect, secrete rhBMP-2 under exogenous regulation, and induced bone formation by paracrine effect, with no adverse or immune response to the transplanted capsules.
Lin, Hong-Ru; Yeh, Yu-Jen	2004	Design of an alginate/hydroxyapatite (HAP) composite scaffolds with a well-interconnected porous structure. The HAP showed a better cell attachment than pure alginate when rat osteosarcoma UMR106 cells were used.
Buket Basmanav, F.; Kose, Gamze T.; Hasirci, Vasif	2008	Design of a 3D tissue-engineering scaffold capable of sequentially delivering two bone morphogenetic proteins (BMP), BMP-2 and BMP-7. The microspheres carrying the growth factors enhanced osteogenic differentiation to a higher degree than with their single administration.
Grellier, Maritie; et al.	2009	Immobilization of human osteoprogenitors (HOP) from bone marrow mesenchymal stem cells alone or together with human umbilical vein endothelial cells (HUVEC) inside irradiated, oxidized and RGD-grafted alginate microspheres. Shown that the gene expression of alkaline phosphatase and osteocalcin were upregulated, and that the VEGF secretion was increased. When implanted in a bone defect, mineralization was observed inside and around the implanted microspheres containing the immobilized cells.
López-Morales, Yaiza; et al.	2010	Investigation of the use of bone morphogenetic proteins (rhBMP-2, rhBMP-4) alone or in combination with cells delivered in a calcium alginate gel for the treatment of osteochondral defects. In the results was observed that rhBMP-2 showed better restoration of subchondral bone while rhBMP-4 has a superior efficiency for hyaline cartilage repair.
Kanczler, Janos M.; et al.	2010	Determination of the enhanced bone regenerative capability in a critical sized femur defect of human bone marrow stromal cells (HBMSC) when seeded and delivered with an Alginate-VEGF165/PDLLA-BMP-2 scaffolds.
Vuong, Jenny; Hellmich, Christian	2011	Analysis of data from bone drying, demineralization, and deorganification tests, collected over a time span of more than 80 years, revealing that exists a unique bilinear relationship between organic concentration and mineral concentration, across different species, organs, and age groups, from early childhood to old age: During organ growth, the mineral concentration increases linearly with the organic concentration (which increases during fibrillogenesis), while from adulthood on, further increase of the mineral concentration is accompanied by a decrease in organic concentration.
Kolambkar, Yash M.; et al.	2011	Introduction of a hybrid growth factor delivery system that consists of an electrospun nanofiber mesh tube for guiding bone regeneration combined with peptide-modified alginate hydrogel injected inside the tube for sustained growth factor release. The results indicated that the hybrid alginate/nanofiber mesh system is a promising growth factor delivery strategy for the repair of challenging bone injuries.
Oryan, A; Alidadi, S; Moshiri, A	2013	Review of the current information of orthopaedic surgeons and investigators working in the field of bone healing.
Allison, Daniel C.; et al.	2013	Consolidation of the recent literature regarding the use of bone grafts for the treatment of surgically created, non-structural, cavitary bone defects in children. Proving that no single option has been demonstrated to be superior to each other yet.
Bendtsen, Stephanie T.; Wei, Mei	2015	Development of an injectable alginate hydrogel with a gelation time ranging from 5-10 minutes by varying the concentrations of phosphate and calcium involved in the gelation process. Proving that this novel fabrication process of an injectable hydrogel system with components necessary for promoting enhanced bone regeneration and great osteoconductivity were viable for host-implant integration.
Venkatesan, Jayachandran; et al.	2015	Overview of alginate preparation and its applications towards bone tissue engineering.
Yan, Jingxuan; et al.	2016	Development of an injectable and biodegradable alginate-based composite gel scaffolds doubly integrated with hydroxyapatite (HAp) and gelatin microspheres (GMs) cross-linked via in situ release of calcium cations. The results demonstrated that the HAp and GMs doubly integrated alginate-based gel scaffolds, have suitable physical performance and bioactive properties, and a potential opportunity to be used for bone tissue engineering, because when osteoblasts where encapsulated combination with TH, the gel scaffold exhibited beneficial effects on osteoblast activity.

The table 8 shows all the articles and original studies used to complete the subsection of the review “The use of alginate for Nerve tissue engineering”. The table is organized according to their research group, year of publication and a summary of the most relevant results.

Table 8. Articles used in the section Alginate for Nerve tissue engineering

Research Group	Year of publication	Summary
Lundborg, Göran; Longo, Frank M.; Varon, Silvio	1982	Regenerating of the proximal stump of a transected rat sciatic nerve through a cylindrical silicone chamber across a 10 mm gap to the distal stump. The fluid filling such in vivo chambers contains trophic factors that ensure in vitro survival and growth of sensory neurons from rodent dorsal root ganglia.
Suzuki, Yoshihisa; et al.	1999	Development of an artificial nerve guide composed of biodegradable freeze-dried alginate gel covered by polyglycolic acid mesh. Using a 50-mm gap cat sciatic nerve model, the results showed functional reinnervation of motor and sensory nerves after 13 weeks, the recovery of compound muscle action potential (CMAP) and somatosensory evoked potential (SEP) and the development of new fasciculi nerves.
Suzuki, Kyoko; et al.	1999	Study of the capability of alginate gel to promote nerve regeneration in the severed spinal cord of adult mammals. The results showed that 45 days after implanting alginate gel in the resection gap of the T9-T10 spinal cord of Wistar rats, myelinated and unmyelinated axons regenerated throughout the gap with remaining alginate gel was observed. The elongated axons established electrophysiologically functional projections across the gap.
Kataoka, Kazuya; et al.	2004	Examination, at early stages after surgery, the outgrowth of regenerating axons and reactions of astrocytes at the stump of transected spinal cord in young rats after the implantation of alginate gels. The results suggested that alginate contributed to reducing the barrier composed of connective tissues and reactive astrocytic processes, while serving as a scaffold for the outgrowth of regenerating axons and elongation of astrocytic processes.
Prang, Peter; et al.	2006	Introduction of alginate-based highly anisotropic capillary hydrogels (ACH) into an axon outgrowth assay in vitro and adult rat spinal cord lesions in vivo, to assess its capacity to promote directed axon regeneration. The results after the implantation into acute cervical spinal cord lesions in adult rats, proved that alginate-based ACH was integrated into the spinal cord parenchyma without major inflammatory responses, maintaining their anisotropic structure and inducing directed axon regeneration across the artificial scaffold.
Suzuki, Yoshihisa; et al.	2016	Use of alginate gel without a tubular structure as an artificial nerve graft for digital nerve repair and the evaluation of peripheral nerve regeneration in clinical trials. In 2 patients, a gap due to digital nerve injury was bridged with controlled-release heparin/alginate gel combined with basic fibroblast growth factor, and restoration of the sensory function was serially evaluated.
Liu, Shengwen; et al.	2017	Study in which the combination of an alginate biomaterial with linear channels with transplantation of Schwann cells within and beyond the lesion site and injection of a regulatable vector for the transient expression of brain-derived neurotrophic factor (BDNF) proved that only with the full combination axons extend across the lesion site and that expression of BDNF beyond 4 weeks does not further increase the number of regenerating axons.
Salehi, Majid; et al.	2019	Use of alginate/chitosan (alg/chit) hydrogel for the transplantation of olfactory ectomesenchymal stem cells (OE-MSCs) to promote peripheral nerve regeneration. The results of injecting the alg/chi hydrogel into a 3-mm sciatic nerve defect of Wistar rats showed that utilizing this hydrogel with OE-MSCs to the sciatic nerve defect enhance regeneration compared to the control group and hydrogel without cells.

The table 9 shows all the articles and original studies used to complete the subsection of the review “The use of alginate for Pancreatic tissue engineering”. The table is organized according to their research group, year of publication and a summary of the most relevant results.

Table 9. Articles used in the section Alginate for Pancreatic tissue engineering.

Research Group	Year of publication	Summary
Lim, Franklin; Sun, Anthony M.	1980	Single implantation of microencapsulated islets into rats with streptozotocin-induced diabetes corrected the diabetic state for 2 to 3 weeks. The microencapsulated islets remained morphologically and functionally intact throughout long-term culture studies lasting over 15 weeks
Soon-Shiong, P.; et al.	1994	Reports for insulin independence in a type 1 diabetic patient after encapsulated islet transplantation. Encapsulated human islets were injected intraperitoneally in a diabetic patient with a functioning kidney graft. Insulin independence with tight glycaemic control was demonstrated 9 months after the procedure.
De Vos, P.; et al.	1997	Investigation about the purification of alginate to improve the biocompatibility of alginate-polylysine microcapsules. The microcapsules prepared from crude or purified alginate were implanted in the peritoneal cavity of normoglycaemic AO-rats. The purified alginate microcapsules show an improved biocompatibility and immunoprotective properties.
Tatarkiewicz, K; et al.	2001	This study aimed to assess a response of microencapsulated rat islets to a meal challenge after being transplanted intraperitoneally into diabetic mice. The results showed that the delivery of C peptide and the accompanying insulin were delayed by restrictions of the capsules and the peritoneal location. However, this delay in reaching peripheral target organs does not prevent microencapsulated grafts from efficiently clearing glucose after a meal.
Calafiore, Riccardo; et al.	2006	Phase 1 pilot clinical trial of microencapsulated Time-related decline of human islet allograft (TX) into 10 non-immunosuppressed patients with type 1 diabetes.
Yang, Jia; et al.	2015	Use of inkjet printing to pattern biogenic nanoparticles, i.e., mutant tobacco mosaic virus (TMV), with different spot sizes to support the formation of multicellular clusters by pancreatic progenitor cells (PPCs). A TMV particle patten was successfully achieved with variable features and sizes by adjusting the surface wettability and printing speed. The PPCs stably attached, proliferated on top of the TMV-RGD support, resulting in the formation of uniform and confluent PPC clusters. The aggregated PPCs also maintained their multipotency and were positive for E-cadherin.
Duin, Sarah; et al.	2019	Combination of islet encapsulation with 3D extrusion bioprinting, to produce macroporous hydrogel constructs with embedded pancreatic islets, using clinically approved ultrapure alginate and methylcellulose (Alg/MC). The embedded islets continuously produced insulin and glucagon throughout the observation and still reacted to glucose stimulation albeit to a lesser degree than control islets.

The table 10 shows all the articles and original studies used to complete the subsection of the review “The use of alginate for Liver tissue engineering”. The table is organized according to their research group, year of publication and a summary of the most relevant results.

Table 10. Articles used in the section Alginate for Liver tissue engineering.

Research Group	Year of publication	Summary
Gutsche, Annie Tang; et al.	1996	Engineering of a porous carbohydrate-derivatized substrate for hepatocyte culture. The carbohydrate-derivatized porous substrates proved to be useful for large-scale culture of hepatocytes, toxicology screening and for use in a liver assist device.
Yang, Jun; et al.	2001	Preparation of porous scaffolds of alginate/galactosylated chitosan (ALG/GC) sponges by lyophilization for liver-tissue engineering. The results showed that the hepatocytes in ALG/GC sponges had higher cell attachment and viability. Improvements in spheroid formation and long-term liver-specific functions of the immobilized hepatocyte were also observed.

Table 10. Part II

Research Group	Year of publication	Summary
Dvir-Ginzberg, Mona; et al.	2003	This work addressed cell seeding of hepatocytes and its distribution within porous alginate scaffolds. The results of the study highlighted the importance of cell density on the hepatocellular functions of three-dimensional hepatocyte constructs.
Chen, Feng; et al.	2012	Report of a Liver tissue engineering scaffold derived from oxidized alginate covalently cross-linked galactosylated chitosan via Schiff base reaction, without employing any extraneous chemical cross-linking agent. The results showed that the scaffolds displayed highly porous surfaces and interconnected pore structure in the internal structure. Biocompatibility studies showed that the hepatocytes cultured on the scaffolds had a typical spheroidal morphology, formed multi-cellular aggregates, and presented perfect integration with the scaffolds.
Rebelo, Sofia P.; et al.	2015	Presentation of a three-dimensional (3D) strategy for the differentiation of HepaRG based on alginate microencapsulation of cell spheroids and culture in dimethyl sulfoxide (DMSO)-free conditions. The resulting model was suitable for toxicological applications, as it allows high throughput analysis of multiple compounds in a DMSO-free setting. Also, due to the high xenobiotic metabolism and maintenance of biosynthetic functions, this model might be broadened to understand liver physiology and for bioartificial liver applications.
Tong, Xiao-Fang; et al.	2018	Development of a biodegradable and injectable in situ hydrogel formed by glycyrrhizin (GL), alginate (Alg), and calcium (Ca) for three-dimensional (3D) cell culture. The results suggested that the hydrogel was homogenous with stable structure and well viscoelasticity, also human hepatoma HepG2 cells cultured in hydrogels showed well morphology and could maintain the viability, proliferation, and liver function for longer periods of time. Furthermore, the hydrogel improved the mRNA expression of cytochrome P450.
Pasqua, Mattia; et al.	2020	Encapsulation of HepaRG cells (precursors of hepatocyte-like cells) in 1.5% alginate beads without pre-forming spheroids. The results showed that cells self-rearranged as aggregates within the beads and adequately differentiated over time, in the absence of any differentiating factors classically used. On day 14 post-encapsulation, cells displayed a wide range of hepatic features necessary for the treatment of a patient in acute liver failure.

5. Conclusion

In conclusion, although alginate has become rapidly a very useful tool as biomaterial for tissue engineering due its great versatility, ability to form hydrogels that provide three-dimensional microenvironments that mimics the ECM, and has allowed to obtain a better understanding of how the tissues and organs regenerate, it is undeniable that organ transplantation remains the most successful therapy for end-stage diseases, even though tissue engineering could be a much more effective therapy. This could be because, although a large number of *in vitro* studies are carried out, the high requirements of medical groups, regulations and standards on tissue engineering products are leading to the reductions and delay in the number of clinical trials, especially with complex tissues such as heart and liver which are still far from being translated clinically. Despite all this, clinical trials with patients with injuries in tissues such as cartilage, bone, or skin have already underway, providing better prospects for the future progress towards better clarification of how to repair and regenerate any organ system.

6. Future considerations

Future lines of research on alginate in tissue engineering may focus on promoting and making the most of new scaffold creation techniques such as 3D-bioprinting to accelerate the process of developing and creation of tail-made hydrogels, and also in boosting the test of injectable hydrogels in humans to enable the evolution of biomedicine towards less invasive therapies.

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<https://doi.org/10.1021/bm060099n>
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ANNEX I

Table 2. Total of articles and original works used to carry out the thesis

Author	Title	Journal	Volume	Number	Year	Booktitle
Aarstad, Olav Andreas; et al	Biosynthesis and Function of Long Guluronic Acid-Blocks in Alginate Produced by <i>Azotobacter vinelandii</i>	Biomacromolecules			2019	
Agulhon, Pierre; et al.	Structure of alginate gels: Interaction of diuronate units with divalent cations from density functional calculations	Biomacromolecules	13	6	2012	
Ahmed, Enas M.	Hydrogel: Preparation, characterization, and applications: A review	Journal of Advanced Research	6	2	2015	
AlgiPharma AS	A Phase 2b Randomised, Placebo Controlled Study of OligoG in Patients With Cystic Fibrosis	ClinicalTrials.gov			2019	
Allison, Daniel C.; et al.	Bone grafting alternatives for cavitary defects in children	Current Orthopaedic Practice	24	3	2013	
Alsberg, E.; et al.	Regulating bone formation via controlled scaffold degradation	Journal of Dental Research	82	11	2003	
Andersen, Therese; et al.	Ionically gelled alginate foams: Physical properties controlled by operational and macromolecular parameters	Biomacromolecules	13	11	2012	
Andersen, Therese; et al.	Ionically gelled alginate foams: Physical properties controlled by type, amount and source of gelling ions	Carbohydrate Polymers			2014	
Andrei, Mihaela-Cristina; et al.	Surgical Treatment in Acute Phase of Severe Burns - a Comprehensive Approach	Medicina Moderna - Modern Medicine	25	1	2018	
Anversa, Piero; et al.	Concise Review: Stem Cells, Myocardial Regeneration, and Methodological Artifacts	STEM CELLS	25	3	2006	
Arlov, Øystein; et al.	The Impact of Chain Length and Flexibility in the Interaction between Sulfated Alginates and HGF and FGF-2	Biomacromolecules	16	11	2015	
Arlov, Øystein; Skjåk-Bræk, Gudmund	Sulfated alginates as heparin analogues: A review of chemical and functional properties		22	5	2017	Molecules
Balakrishnan, Biji; Jayakrishnan, A.	Self-cross-linking biopolymers as injectable in situ forming biodegradable scaffolds	Biomaterials			2005	
Balakrishnan, B.; Lesieur, S.; Labarre, D.; Jayakrishnan, A.	Periodate oxidation of sodium alginate in water and in ethanol-water mixture: A comparative study	Carbohydrate Research	340	7	2005	
Bencherif, Sidi A.; et al.	Injectable preformed scaffolds with shape-memory properties	Proceedings of the National Academy of Sciences of the United States of America			2012	
Bendtsen, Stephanie T.; Wei, Mei	Synthesis and characterization of a novel injectable alginate-collagen-hydroxyapatite hydrogel for bone tissue regeneration	Journal of Materials Chemistry B	3	15	2015	
Bhattarai, Narayan; Zhang, Miqin	Controlled synthesis and structural stability of alginate-based nanofibers	Nanotechnology			2007	
BioLineRx, L.	Safety and feasibility of the injectable BL-1040 implant				2009	Study NCT00557531
Bochenek, Matthew A.; et al	Alginate encapsulation as long-term immune protection of allogeneic pancreatic islet cells transplanted into the omental bursa of macaques	Nature Biomedical Engineering			2018	
Bonino, Christopher A.; et al.	Electrospinning alginate-based nanofibers: From blends to crosslinked low molecular weight alginate-only systems	Carbohydrate Polymers			2011	
Bouhadir, Kamal H.; et al.	Degradation of partially oxidized alginate and its potential application for tissue engineering	Biotechnology Progress			2001	
Brus, Jiri; et al.	Structure and Dynamics of Alginate Gels Cross-Linked by Polyvalent Ions Probed via Solid State NMR Spectroscopy	Biomacromolecules	18	8	2017	
Bui, Anh L.; Horwich, Tamara B.; Fonarow, Gregg C.	Epidemiology and risk profile of heart failure		8	1	2011	Nature Reviews Cardiology
Buitelaar, R.; Bucke, C.; Tramper, R.; Wijffels, R.	Immobilized Cells: Basics and Applications: Basics and Applications				1996	

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Table 2. Part II						
Buket Basmanav, F.; Kose, Gamze T.; Hasirci, Vasif	Sequential growth factor delivery from complexed microspheres for bone tissue engineering	Biomaterials	29	31	2008	
Calafiore, Riccardo; et al.	Microencapsulated pancreatic islet allografts into nonimmunosuppressed patients with type 1 diabetes	Diabetes Care	29	1	2006	
Catanzano, Ovidio; et al.	Macroporous alginate foams crosslinked with strontium for bone tissue engineering	Carbohydrate Polymers			2018	
Chan, Lai Wah; Lee, Huey Ying; Heng, Paul W. S.	Mechanisms of external and internal gelation and their impact on the functions of alginate as a coat and delivery system	Carbohydrate Polymers	63	2	2006	
Chaturvedi, Kiran; et al.	Sodium alginate in drug delivery and biomedical areas				2019	Natural Polysaccharides in Drug Delivery and Biomedical Applications
Chaudhuri, Ovijit; et al.	Substrate stress relaxation regulates cell spreading	Nature Communications	6	1	2015	
Chaudhuri, Ovijit; et al.	Hydrogels with tunable stress relaxation regulate stem cell fate and activity	Nature Materials	15	3	2016	
Chen, Feng; et al.	Preparation and characterization of oxidized alginate covalently cross-linked galactosylated chitosan scaffold for liver tissue engineering	Materials Science and Engineering C	32	2	2012	
Cho, Wan Jin; Oh, Heang; Lee, Jin Ho	Alginate Film as a Novel Post-Surgical Tissue Adhesion Barrier	Journal of Biomaterials Science	21		2010	
Choudhary, Soumitra; Bhatia, Surita R.	Rheology and nanostructure of hydrophobically modified alginate (HMA) gels and solutions	Carbohydrate Polymers	87	1	2012	
Choudhary, Soumitra; et al.	Hydrophobically modified alginate for extended release of pharmaceuticals	Polymers for Advanced Technologies	29	1	2018	
Coleman, Robert J.; et al.	Phosphorylation of alginate: Synthesis, characterization, and evaluation of in vitro mineralization capacity	Biomacromolecules	12	4	2011	
Colinet, I.; Dulong, V.; Mocanu, G.; Picton, L.; Le Cerf, D.	New amphiphilic and pH-sensitive hydrogel for controlled release of a model poorly water-soluble drug	European Journal of Pharmaceutics and Biopharmaceutics	73	3	2009	
Copp, D. Harold; Shim, S. S.	The homeostatic function of bone as a mineral reservoir	Oral Surgery, Oral Medicine, Oral Pathology	16	6	1963	
Costa, Maria J.; et al.	Physicochemical properties of alginate-based films: Effect of ionic crosslinking and mannuronic and guluronic acid ratio	Food Hydrocolloids			2018	
Dahlmann, J; et al.	Fully defined in situ cross-linkable alginate and hyaluronic acid hydrogels for myocardial tissue engineering	Biomaterials			2013	
Dar, Ayelet; Shachar, Michal; Leor, Jonathan; Cohen, Smadar	Optimization of cardiac cell seeding and distribution in 3D porous alginate scaffolds	Biotechnology and Bioengineering	80	3	2002	
De Boisseson, M. Rastello; et al.	Physical alginate hydrogels based on hydrophobic or dual hydrophobic/ionic interactions: Bead formation, structure, and stability	Journal of Colloid and Interface Science	273	1	2004	
Deramos, C. M.; Irwin, A. E.; Nauss, J. L.; Stout, B. E.	¹³ C NMR and molecular modeling studies of alginic acid binding with alkaline earth and lanthanide metal ions	Inorganica Chimica Acta			1997	
De Vos, P.; et al.	Improved biocompatibility but limited graft survival after purification of alginate for microencapsulation of pancreatic islets	Diabetologia	40	3	1997	
De Vos, Paul; De Haan, Bart; Van Schilfgaarde, Reinout	Effect of the alginate composition on the biocompatibility of alginate-polylysine microcapsules	Biomaterials			1997	
Donati, Ivan; et al.	New hypothesis on the role of alternating sequences in calcium-alginate gels	Biomacromolecules	6	2	2005	
Dong, Zhanfeng; Wang, Qun; Du, Yumin	Alginate/gelatin blend films and their properties for drug-controlled release	Journal of Membrane Science	280		2006	
Drury, Jeanie L.; Mooney, David J.	Hydrogels for tissue engineering: Scaffold design variables and applications	Biomaterials	24	24	2003	
Drury, Jeanie L.; Dennis, Robert G.; Mooney, David J.	The tensile properties of alginate hydrogels	Biomaterials			2004	
Duin, Sarah; et al.	3D Bioprinting of Functional Islets of Langerhans in an Alginate/Methylcellulose Hydrogel Blend	Advanced Healthcare Materials	8	7	2019	

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Table 2. Part III						
Dvir-Ginzberg, Mona; Gamlieli-Bonshtein, Iris; Agbaria, Riad; Cohen, Smadar	Liver Tissue Engineering within Alginate Scaffolds: Effects of Cell-Seeding Density on Hepatocyte Viability, Morphology, and Function	Tissue Engineering	9	4	2003	
Fan, Lihong; et al.	Synthesis and anticoagulant activity of sodium alginate sulfates	Carbohydrate Polymers	83	4	2011	
Fischer, F. G.; Dørfel, Helmunt	Die Polyuronsäuren der Braunalgen (Kohlenhydrate der Algen I)	Hoppe-Seyler's Zeitschrift für Physiologische Chemie	302		1955	
Fischl, Richard; et al.	The cell-wall active mannuronan C5-epimerases in the model brown alga Ectocarpus: From gene context to recombinant protein	Glycobiology			2016	
Freeman, Inbar; Kedem, Alon; Cohen, Smadar	The effect of sulfation of alginate hydrogels on the specific binding and controlled release of heparin-binding proteins	Biomaterials	29	22	2008	
Frey, Norbert; et al.	Intracoronary delivery of injectable bioabsorbable scaffold (IK-5001) to treat left ventricular remodeling after ST-elevation myocardial infarction: A first-in-man study	Circulation: Cardiovascular Interventions	7	6	2014	
Gajendiran, Mani; et al.	Conductive biomaterials for tissue engineering applications				2017	Journal of Industrial and Engineering Chemistry
Gilchrist, T.; Martin, A. M.	Wound treatment with Sorbsan - an alginate fibre dressing	Biomaterials			1983	
Goh, Cheong Hian; Heng, Paul Wan Sia; Chan, Lai Wah	Alginates as a useful natural polymer for microencapsulation and therapeutic applications	Carbohydrate Polymers	88	1	2012	
Gombotz, Wayne R.; Wee, Siow Fong	Protein release from alginate matrices	Advanced Drug Delivery Reviews	64	SUPPL.	2012	
Gomez, C. G.; Rinaudo, M.; Villar, M. A.	Oxidation of sodium alginate and characterization of the oxidized derivatives	Carbohydrate Polymers			2007	
Gong, Ying; et al.	Cytotoxicity and antiviral activity of calcium alginate fibers and zinc alginate fibers		152-153		2011	Advanced Materials Research
Gonzalez-Fernandez, Tomas; et al.	Gene Delivery of TGF- β 3 and BMP2 in an MSC-Laden Alginate Hydrogel for Articular Cartilage and Endochondral Bone Tissue Engineering	Tissue Engineering Part A	22	9-10	2016	
Grellier, Maritie; et al.	The effect of the co-immobilization of human osteoprogenitors and endothelial cells within alginate microspheres on mineralization in a bone defect	Biomaterials	30	19	2009	
Groves, A. R.; Lawrence, J. C.	Aliginat dressing as a donor site haemostat	Annals of the Royal College of Surgeons of England			1986	
Gutsche, Annie Tang; et al.	Engineering of a sugar-derivatized porous network for hepatocyte culture	Biomaterials			1996	
Hammett, Frederick S.	II. Changes in the Calcium, Magnesium, and phosphorus of bone during growth.	A BIOCHEMICAL STUDY OF BONE GROWTH .			1925	
Han, Donling; Yan, Lifeng; Chen, Wufen; Li, Wan	Preparation of chitosan/graphene oxide composite film with enhanced mechanical strength in the wet state	Carbohydrate Polymers	83	2	2011	
Haug, Arne; et al.	The Solubility of Alginate at Low pH.	Acta Chemica Scandinavica			1963	
Haug, Arne	Composition and Properties of Alginates	Norwegian Institute of Seaweed Research			1964	
Haug, Arne; et al.	A Study of the Constitution of Alginic Acid by Partial Acid Hydrolysis.	Acta Chemica Scandinavica			1966	
Haug, Arne; et al.	Studies on the Sequence of Uronic Acid Residues in Alginic Acid.	Acta Chemica Scandinavica	21		1967	
Haug, Arne; Larsen, Bjørn	Biosynthesis of alginate. Epimerisation of d-mannuronic to l-guluronic acid residues in the polymer chain	BBA - General Subjects	192	3	1969	
Haug, Arne; Larsen, Bjørn; Smidsrød, Olav	Uronic acid sequence in alginate from different sources	Carbohydrate Research			1974	
Hecht, Hadas; Srebnik, Simcha	Structural Characterization of Sodium Alginate and Calcium Alginate	Biomacromolecules	17	6	2016	
Hegge, Anne Bee; Andersen, T.; Melvik, J. E.; Kristensen, S.; Tønnesen, H. H.	Evaluation of novel alginate foams as drug delivery systems in antimicrobial photodynamic therapy (aPDT) of infected wounds - An in vitro study: Studies on curcumin and curcuminoides XL	Journal of Pharmaceutical Sciences			2010	

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Hegge, Anne Bee; et al.	Formulation and bacterial phototoxicity of curcumin loaded alginate foams for wound treatment applications: Studies on curcumin and curcuminoides XLII	Journal of Pharmaceutical Sciences			2011	
Helgerud, Trond; et al.	Alginates				2009	Food Stabilisers, Thickeners and Gelling Agents
Kaklamani, Georgia; et al.	Mechanical properties of alginate hydrogels manufactured using external gelation	Journal of the Mechanical Behavior of Biomedical Materials			2014	
Kamoun, Elbadawy A.; Kenawy, El Refaie S.; Chen, Xin	A review on polymeric hydrogel membranes for wound dressing applications: PVA-based hydrogel dressings		8	3	2017	Journal of Advanced Research
Kanczler, Janos M.; et al.	The effect of the delivery of vascular endothelial growth factor and bone morphogenic protein-2 to osteoprogenitor cell populations on bone formation	Biomaterials	31	6	2010	
Kanczler, Janos M.; et al.	The effect of the delivery of vascular endothelial growth factor and bone morphogenic protein-2 to osteoprogenitor cell populations on bone formation	Biomaterials	31	6	2010	
Kataoka, Kazuya; et al.	Alginate Enhances Elongation of Early Regenerating Axons in Spinal Cord of Young Rats		10	3-4	2004	Tissue Engineering
Klöck, Gerd; et al.	Production of purified alginates suitable for use in immunisolated transplantation	Applied Microbiology and Biotechnology	40	5	1994	
Klöck, Gerd; et al.	Biocompatibility of mannuronic acid-rich alginates	Biomaterials			1997	
Houben, Roland	An alginate-based hybrid system for growth factor delivery in the functional repair of large bone defects	Biomaterials	32	1	2011	
Koltzenburg, Sebastian; et al.	Polymer chemistry				2017	Polymer Chemistry
Kuo, Catherine K.; Ma, Peter X.	Ionically crosslinked alginate hydrogels as scaffolds for tissue engineering: Part 1. Structure, gelation rate and mechanical properties	Biomaterials	22	6	2001	
Landa, Natali; et al.	Effect of injectable alginate implant on cardiac remodeling and function after recent and old infarcts in rat	Circulation			2008	
Larsen, Bjørn; et al.	Calculation of the Nearest-neighbour Frequencies in Fragments of Alginate from the Yields of Free Monomers after Partial Hydrolysis.	Acta Chemica Scandinavica			1970	
Lee, Kuen Yong; et al.	Degradation behavior of covalently cross-linked poly(aldehyde guluronate) hydrogels	Macromolecules	33	1	2000	
Lee, Kuen Yong; Bouhadir, Kamal H.; Mooney, David J.	Controlled degradation of hydrogels using multi-functional cross-linking molecules	Biomaterials	25	13	2004	
Lee, Sang Jin; Yoo, James J.; Atala, Anthony	Biomaterials and tissue engineering				2017	Clinical Regenerative Medicine in Urology
Leonard, M.; et al..	Hydrophobically modified alginate hydrogels as protein carriers with specific controlled release properties	Journal of Controlled Release	98	3	2004	
Leor, Jonathan; et al.	Bioengineered cardiac grafts: A new approach to repair the infarcted myocardium?	Circulation			2000	
Leor, Jonathan; et al.	Natali; Feinberg	Micha S.; Konen	Eli; Goitein	Orly; Tsur-Gang	Lea; Cohen	
Leung, Victor; et al.	Postelectrospinning modifications for alginate nanofiber-based wound dressings	Journal of Biomedical Materials Research Part B: Applied Biomaterials	102	3	2014	
Li, Liangbin; et al.	Reexamining the egg-box model in calcium - Alginate gels with X-ray diffraction	Biomacromolecules	8	2	2007	
Li, Xiaoxia; et al.	Preparation of low molecular weight alginate by hydrogen peroxide depolymerization for tissue engineering	Carbohydrate Polymers			2010	
Lim, F.; Sun, A.	Microencapsulated islets as bioartificial endocrine pancreas	Science	210	4472	1980	
Lin, Hong-Ru; Yeh, Yu-Jen	Porous alginate/hydroxyapatite composite scaffolds for bone tissue engineering: Preparation, characterization, and in vitro studies	Journal of Biomedical Materials Research	71B	1	2004	
Linhardt, Robert J.	2003 Claude S. Hudson award address in carbohydrate chemistry. Heparin: Structure and activity				2003	Journal of Medicinal Chemistry
Liu, Shengwen; et al.	Regulated viral BDNF delivery in combination with Schwann	Acta Biomaterialia	60		2017	

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Loh, Qiu Li; Choong, Cleo	Three-dimensional scaffolds for tissue engineering applications: Role of porosity and pore size					2013	Tissue Engineering - Part B: Reviews
López-Morales, Yaiza; et al.	In vivo comparison of the effects of RHBMP-2 and RHBMP-4 in osteochondral tissue regeneration	European Cells and Materials				2010	
Lundborg, Göran; et al.	Nerve regeneration model and trophic factors in vivo	Brain Research	232	1		1982	
Lyn, Tsau-Yen; Hassid, W. Z.	Pathway of Alginic Acid Synthesis in the Pathway Fucus of Alzzinic Acid gardneri in the Marine Alga, Focus gardneri Silva*	The Journal of Biological Chemistry				1966	
Maitra, Jaya; Kumar Shukla, Vivek	Cross-linking in Hydrogels-A Review	American Journal of Polymer Science	2014	2		2014	
Martinsen, A.; Skjåk-Bræk, G.; Smidsrød, O.	Alginate as immobilization material: I. Correlation between chemical and physical properties of alginate gel beads	Biotechnology and Bioengineering	33	1		1989	
Mærk, Mali; et al	Identification of Regulatory Genes and Metabolic Processes Important for Alginate Biosynthesis in <i>Azotobacter vinelandii</i> by Screening of a Transposon Insertion Mutant Library	Frontiers in Bioengineering and Biotechnology				2020	
McKinnon, Daniel D.; Domaille, Dylan W.; Cha, Jennifer N.; Anseth, Kristi S.	Biophysically Defined and Cytocompatible Covalently Adaptable Networks as Viscoelastic 3D Cell Culture Systems	Advanced Materials	26	6		2014	
Mhanna, Rami; et al.	Chondrocyte culture in three-dimensional alginate sulfate	Rissue Engineering	20	09-oct		2014	
Mhanna, Rami; Becher, Jana; Schnabelrauch, Matthias; Reis, Rui L.; Pashkuleva, Iva	Sulfated Alginate as a Mimic of Sulfated Glycosaminoglycans: Binding of Growth Factors and Effect on Stem Cell Behavior	Advanced Biosystems	1	7		2017	
Mi, Fwu Long; Sung, Hsing Wen; Shyu, Shin Shing	Drug release from chitosan-alginate complex beads reinforced by a naturally occurring cross-linking agent	Carbohydrate Polymers				2002	
Michel, Gurvan; Tonon, Thierry; Scornet, Delphine; Cock, J. Mark; Kloreg, Bernard	The cell wall polysaccharide metabolism of the brown alga <i>Ectocarpus siliculosus</i> . Insights into the evolution of extracellular matrix polysaccharides in Eukaryotes	New Phytologist	188	1		2010	
Mikołajczyk, Teresa; Wołowska-Czapnik-Czapnik, Dorota	Multifunctional alginate fibres with anti-bacterial properties	Fibres and Textiles in Eastern Europe				2005	
Moe, Størker; et al.	Calcium alginate gel fibers: Influence of alginate source and gel structure on fiber strength	Journal of Applied Polymer Science	51	10		1994	
Müller, Michael; et al.	Alginate Sulfate–Nanocellulose Bioinks for Cartilage Bioprinting Applications	Annals of Biomedical Engineering	45	1		2017	
Nesic, Aleksandra R.; Seslija, Sanja I.	The influence of nanofillers on physical & chemical properties of polysaccharide-based film intended for food packaging					2017	Food Packaging
Nyvall, Pi; et al.	Characterization of Mannuronan C-5-Epimerase Genes from the Brown Alga <i>Laminaria digitata</i>	Plant Physiology				2003	
Odell, E. W.; Lombardi, T.; Oades, P.	Symptomatic foreign body reaction to haemostatic alginate	British Journal of Oral and Maxillofacial Surgery				1994	
Orive, G.; et al.	Biocompatibility evaluation of different alginates and alginate-based microcapsules	Biomacromolecules	6	2		2005	
Oryan, A.; Alidadi, S.; Moshiri, A.	Current concerns regarding healing of bone defects	Hard Tissue	2	2		2013	
Otterlei, Marit; et al.	Induction of Cytokine Production from Human Monocytes Stimulated with Alginate	Journal of Immunotherapy	10	4		1991	
Oveissi, F.; Naficy, S.; Lee, A.; Winlaw, D. S.; Dehghani, F.	Materials and manufacturing perspectives in engineering heart valves: a review					2020	Materials Today Bio
Park, Jisun; Lee, Su Jeong; Lee, Hwangjae; Park, Su A.; Lee, Jae Young	Three-dimensional cell printing with sulfated alginate for improved bone morphogenetic protein-2 delivery and osteogenesis in bone tissue engineering	Carbohydrate Polymers	196			2018	
Pasqua, Mattia; et al.	HepaRG Self-Assembled Spheroids in Alginate Beads Meet the Clinical Needs for Bioartificial Liver	Tissue Engineering Part A				2020	
Patel, Alpesh; Mequanint, Kibret	Hydrogel Biomaterials					2011	Biomedical Engineering - Frontiers and Challenges
Patil, Smita; Singh, Neetu	Silk fibroin-alginate based beads for human mesenchymal stem cell differentiation in 3D	Biomaterials Science	7	11		2019	

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Table 2. Part VI						
Pawar, Siddhesh N.; Edgar, Kevin J.	Alginate derivatization: A review of chemistry, properties and applications		33	11	2012	Biomaterials
Pérez, René	Ces algues qui nous entourent. Conception actuelle, rôle dans la biosphère, utilisation, culture				1997	Ces algues qui nous entourent. Conception actuelle, rôle dans la biosphère, utilisation, culture
Prang, Peter; et al.	The promotion of oriented axonal regrowth in the injured spinal cord by alginate-based anisotropic capillary hydrogels	Biomaterials	27	19	2006	
Puguan, John Marc C.; Yu, Xiaohong; Kim, Hern	Characterization of structure, physico-chemical properties and diffusion behavior of Ca-Alginate gel beads prepared by different gelation methods	Journal of Colloid and Interface Science	432		2014	
Qi, Meirigeng; et al.	Encapsulation of Human Islets in Novel Inhomogeneous Alginate-Ca ²⁺ /Ba ²⁺ Microbeads: <i>In Vitro</i> and <i>In Vivo</i> Function	Artificial Cells, Blood Substitutes, and Biotechnology	36	5	2008	
Qin, Yimin	Silver-containing alginate fibres and dressings	International Wound Journal	2	2	2005	
Qin, Yimin; Hu, Huiqun; Luo, Aixiang	The conversion of calcium alginate fibers into alginic acid fibers and sodium alginate fibers	Journal of Applied Polymer Science	101	6	2006	
Qin, Yimin	The gel swelling properties of alginate fibers and their applications in wound management				2008	Polymers for Advanced Technologies
Qin, Yimin	Alginate fibres: An overview of the production processes and applications in wound management				2008	Polymer International
Reakasame, Supachai; Boccaccini, Aldo R.	Oxidized Alginate-Based Hydrogels for Tissue Engineering Applications: A Review		19	1	2018	Biomacromolecules
Rebelo, Sofia P.; et al.	HepaRG microencapsulated spheroids in DMSO-free culture: novel culturing approaches for enhanced xenobiotic and biosynthetic metabolism	Archives of Toxicology	89	8	2015	
Remminghorst, Uwe; Rehm, Bernd H. A.	Bacterial alginates: From biosynthesis to applications		28	21	2006	Biotechnology Letters
Rezvanian, Masoud; Mohd Amin, Mohd Cairul Iqbal; Ng, Shio Fern	Development and physicochemical characterization of alginate composite film loaded with simvastatin as a potential wound dressing	Carbohydrate Polymers			2016	
Rhim, Jong Whan	Physical and mechanical properties of water resistant sodium alginate films	LWT - Food Science and Technology			2004	
Rinaudo, Marguerite	Main properties and current applications of some polysaccharides as biomaterials	Polymer International	57	3	2008	
Rioux, L. E.; Turgeon, S. L.; Beaulieu, M.	Characterization of polysaccharides extracted from brown seaweeds	Carbohydrate Polymers	69	3	2007	
Rivero, S.; García, M. A.; Pinotti, A.	Composite and bi-layer films based on gelatin and chitosan	Journal of Food Engineering			2009	
Rokstad, Anne Mari; et al.	Cell-compatible covalently reinforced beads obtained from a chemoenzymatically engineered alginate	Biomaterials			2006	
Ronghua, Huang; Yumin, Du; Jianhong, Yang	Preparation and in vitro anticoagulant activities of alginate sulfate and its quaterized derivatives	Carbohydrate Polymers	52	1	2003	
Rosellini, Elisabetta; Cristallini, Caterina; Barbani, Niccoletta; Vozzi, Giovanni; Giusti, Paolo	Preparation and characterization of alginate/gelatin blend films for cardiac tissue engineering	Journal of Biomedical Materials Research Part A	91A	2	2009	
Russo, R.; Malinconico, M.; Santagata, G.	Effect of cross-linking with calcium ions on the physical properties of alginate films	Biomacromolecules	8	10	2007	
Ruvinov, Emil; Dvir, Tal; Leor, Jonathan; Cohen, Smadar	Myocardial repair: From salvage to tissue reconstruction		6	5	2008	Expert Review of Cardiovascular Therapy
Â Salehi, Majid; et al.	Alginate/chitosan hydrogel containing olfactory ectomesenchymal stem cells for sciatic nerve tissue engineering	Journal of Cellular Physiology	234	9	2019	
Salehi, Majid; et al.	Accelerating healing of excisional wound with alginate hydrogel containing naringenin in rat model	Drug Delivery and Translational Research			2020	
Sapir, Yulia; Kryukov, Olga; Cohen, Smadar	Integration of multiple cell-matrix interactions into alginate scaffolds for promoting cardiac tissue regeneration	Biomaterials	32	7	2011	
Shachar, Michal; Tsur-Gang, Orna; Dvir, Tal; Leor, Jonathan; Cohen, Smadar	The effect of immobilized RGD peptide in alginate scaffolds on cardiac tissue engineering	Acta Biomaterialia	7	1	2011	

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Table 2. Part VII

Shilpa, Anu; Agrawal, S. S.; Ray, Alok R.	Controlled delivery of drugs from alginate matrix					2003	Journal of Macromolecular Science - Polymer Reviews
Sikorski, Pawel; Mo, Frode; Skjåk-Bræk, Gudmund; Stokke, Bjørn T.	Evidence for egg-box-compatible interactions in calcium - Alginate gels from fiber x-ray diffraction	Biomacromolecules	8	7		2007	
Skjåk-Bræk, Gudmund; et al.	Inhomogeneous polysaccharide ionic gels	Carbohydrate Polymers	10	1		1989	
Skjåk-Bræk, Gudmund; Grasdalen, Hans; Larsen, Bjørn	Monomer sequence and acetylation pattern in some bacterial alginates	Carbohydrate Research	154	1		1986	
Slaughter, Brandon V.; et al.	Hydrogels in regenerative medicine	Advanced Materials	21	32-33		2009	
Smidsrød, Olav; Painter, Terence	Effect of periodate oxidation upon the stiffness of the alginate molecule in solution	Carbohydrate Research				1973	
Smidsrød, Olav	Molecular basis for some physical properties of alginates in the gel state	Faraday Discussions of the Chemical Society				1974	
Soon-Shiong, P.; et al.	Insulin independence in a type 1 diabetic patient after encapsulated islet transplantation	The Lancet	343	8903		1994	
Strand, B. L.; Mørch, Y. A.; Skjåk-Bræk, G.	Alginate as immobilization matrix for cells	Minerva Biotechnologica	12	4		2000	
Sutherland, Ian W.	Alginates. In D. Byron (Ed.), Biomaterials: Novel materials from biological sources.					1991	
Suzuki, Kyoko; et al.	Regeneration of transected spinal cord in young adult rats using freeze-dried alginate gel	NeuroReport				1999	
Suzuki, Yoshihisa; et al.	Cat peripheral nerve regeneration across 50 mm gap repaired with a novel nerve guide composed of freeze-dried alginate gel	Neuroscience Letters	259	2		1999	
Suzuki, Yoshihisa; et al.	Nontubulation repair of peripheral nerve gap using heparin/alginate gel combined with b-FGF	Plastic and Reconstructive Surgery - Global Open	4	1		2016	
Tait, Michael	Biomaterials: Novel materials from biological sources	Bioresource Technology	43	1		1993	
Tam, Susan K.; et al.	Factors influencing alginate gel biocompatibility	Journal of Biomedical Materials Research - Part A				2011	
Tatarkiewicz, K.; et al.	C-peptide responses after meal challenge in mice transplanted with microencapsulated rat islets	Diabetologia	44	5		2001	
Thu, Hnin Ei; Zulfakar, Mohd Hanif; Ng, Shioh Fern	Alginate based bilayer hydrocolloid films as potential slow-release modern wound dressing	International Journal of Pharmaceutics				2012	
Thygesen, Kristian; et al	Fourth universal definition of myocardial infarction (2018)	European Heart Journal				2019	
Tong, Xiao-Fang; et al.	Injectable hydrogels based on glycyrrhizin, alginate, and calcium for three-dimensional cell culture in liver tissue engineering	Journal of Biomedical Materials Research Part A	106	12		2018	
Venkatesan, Jayachandran; et al.	Alginate composites for bone tissue engineering: A review		72			2015	International Journal of Biological Macromolecules
Vold, Inger Mari Nygård; Kristiansen, Kåre A.; Christensen, Bjørn E.	A study of the chain stiffness and extension of alginates, in vitro epimerized alginates, and periodate-oxidized alginates using size-exclusion chromatography combined with light scattering and viscosity detectors	Biomacromolecules				2006	
Vuong, Jenny; Hellmich, Christian	Bone fibrillogenesis and mineralization: Quantitative analysis and implications for tissue elasticity	Journal of Theoretical Biology	287	1		2011	
Wang, Lizhe Z.; Liu, Li; Holmes, Justin; Kerry, John F.; Kerry, Joe P.	Assessment of film-forming potential and properties of protein and polysaccharide-based biopolymer films	International Journal of Food Science & Technology	42	9		2007	
Wang, Ting; He, Nongyue	Preparation, characterization and applications of low-molecular-weight alginate & oligochitosan nanocapsules	Nanoscale	2	2		2010	
Williams, D. F.	Definitions in biomaterials. Progress in biomedical engineering.		4			1987	
Williams, David F.	On the mechanisms of biocompatibility	Biomaterials				2008	
Wu, Zongmei; Fan, Weixi; Cai, Xiaoming; Zhu, Chunli	Research progress of alginate in biomedicine	IOP Conference Series: Materials Science and Engineering	729			2020	

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Table 2. Part VIII

Wu, Jie; Wu, Zongmei; Zhang, Ruling; Yuan, Shichao; Lu, Qingliang; Yu, Yueqin	Synthesis and micelle properties of the hydrophobic modified alginate	International Journal of Polymeric Materials and Polymeric Biomaterials	66	14	2017	
Yan, Jingxuan; et al.	Injectable alginate/hydroxyapatite gel scaffold combined with gelatin microspheres for drug delivery and bone tissue engineering	Materials Science and Engineering C	63		2016	
Yang, Jun; Chung, Teak Woong; Nagaoka, Masato; Goto, Mitsuaki; Cho, Chong-Su; Akaike, Toshihiro	Hepatocyte-specific porous polymer-scaffolds of alginate/galactosylated chitosan sponge for liver-tissue engineering		23		2001	Biotechnology Letters
Yang, Ji Sheng; Xie, Ying Jian; He, Wen	Research progress on chemical modification of alginate: A review				2011	Carbohydrate Polymers
Yang, Jia; Zhou, Fang; Xing, Rubo; Lin, Yuan; Han, Yanchun; Teng, Chunbo; Wang, Qian	Development of large-scale size-controlled adult pancreatic progenitor cell clusters by an inkjet-printing technique	ACS Applied Materials and Interfaces	7	21	2015	
Yao, Bolong; Ni, Caihua; Xiong, Cheng; Zhu, Changping; Huang, Bo	Hydrophobic modification of sodium alginate and its application in drug-controlled release	Bioprocess and Biosystems Engineering	33	4	2010	
Ye, Yuanfeng; Zhang, Xiaojuan; Deng, Xiang; Hao, Lingyun; Wang, Wei	Modification of alginate hydrogel films for delivering hydrophobic kaempferol	Journal of Nanomaterials			2019	
You, Fu; Wu, Xia; Chen, Xiongbiao	3D printing of porous alginate/gelatin hydrogel scaffolds and their mechanical property characterization	International Journal of Polymeric Materials and Polymeric Biomaterials	66	6	2017	
Zammaratti, Prisca; Jaconi, Marisa	Cardiac tissue engineering: Regeneration of the wounded heart		15	5	2004	Current Opinion in Biotechnology
Zhao, Huiru; Heindel, Ned D.	Determination of Degree of Substitution of Formyl Groups in Polyaldehyde Dextran by the Hydroxylamine Hydrochloride Method	Pharmaceutical Research: An Official Journal of the American Association of Pharmaceutical Scientists			1991	
Zhao, Xue; Li, Bafang; Xue, Changhu; Sun, Liping	Effect of molecular weight on the antioxidant property of low molecular weight alginate from Laminaria japonica	Journal of Applied Phycology	24	2	2012	
Zilberman, Yoram; Turgeman, Gadi; Pelled, Gadi; Xu, Nong; Moutsatsos, Ioannis K.; Hortelano, Gonzalo; Gazit, Dan	Polymer-encapsulated engineered adult mesenchymal stem cells secrete exogenously regulated rhBMP-2, and induce osteogenic and angiogenic tissue formation	Polymers for Advanced Technologies	13	10-12	2002	

Figure 2.2: Representation of the effect of ManC5-Es on alginate structures

